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# MANAGING SOIL MICROBIAL COMMUNITIES WITH ORGANIC AMENDMENTS TO PROMOTE SOIL AGGREGATE FORMATION AND PLANT HEALTH

Shawn T. Lucas
*University of Kentucky*, slucas_2@yahoo.com

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MANAGING SOIL MICROBIAL COMMUNITIES WITH ORGANIC
AMENDMENTS TO PROMOTE SOIL AGGREGATE FORMATION AND PLANT
HEALTH

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DISSERTATION

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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
Shawn T. H. Lucas

Lexington, KY

Co-Directors:  Dr. Elisa M. D'Angelo, Professor of Soil Science
and        Dr. Mark A. Williams, Professor of Horticulture

Lexington, Kentucky

ABSTRACT OF DISSERTATION

MANAGING SOIL MICROBIAL COMMUNITIES WITH ORGANIC
AMENDMENTS TO PROMOTE SOIL AGGREGATE FORMATION AND PLANT
HEALTH

The effects of managing soil with organic amendments were examined with respect to soil microbial community dynamics, macroaggregate formation, and plant physio-genetic responses. The objective was to examine the possibility of managing soil microbial communities via soil management, such that the microbial community would provide agronomic benefits. In part one of this research, effects of three amendments (hairy vetch residue, manure, compost) on soil chemical and microbial properties were examined relative to formation of large macroaggregates in three different soils. Vetch and manure promoted fungal proliferation (measured via two biomarkers: fatty acid methyl ester 18:2ω6c and ergosterol) and also stimulated the greatest macroaggregate formation. In part two of this research, effects of soil management (same amendments as above, inorganic N fertilization, organic production) on soil chemical and microbial properties were examined relative to the expression of nitrogen assimilation and defense response genes in tomato (*Solanum lycopersicum* L.). Soil management affected expression of a nitrogen assimilation gene (*GS1*, glutamine synthetase) and several defense-related genes. The *GS1* gene was downregulated with inorganic N fertilization, expression of the pathogenesis-related *PR1b* gene (which codes for the pathogenesis-related PR1b protein) was increased in plants grown in soil amended with compost, vetch, and N fertilizer, and expression of three other defense-related genes coding for chitinase (*ChiB*), osmotin (*Osm*), and β-1,3-glucanase (*GluA*) were decreased in plants from soil amended with manure and in plants from the organically managed soil. Differential expression of defense-related genes was inversely related to the relative abundance of Gram-negative bacteria. The relative abundance of the 18:1ω7c Gram-negative bacterial biomarker was greatest in manure treated soil and in organically managed soil (which recieves seasonal manure applications). These treatments also had the lowest expression of *ChiB*, *Osm*, and *GluA*, leading to speculation that manure, through increases in Gram-negative bacteria, may have suppressed populations of soil organisms that induce a defense response in plants, possibly allowing for less-stressed plants. Outcomes of this research may be useful for those interested in developing

management strategies for maintaining or improving soil structure as well as those interested in understanding management effects plant physio-genetic responses.

Shawn T.H. Lucas

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Student's Signature

May 30, 2013

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Date

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By

Shawn T. H. Lucas


Dr. Elisa M. D'Angelo

_____

Co-Director of Dissertation

Dr. Mark A. Williams

_____

Co-Director of Dissertation

Dr. Mark S. Coyne

_____

Director of Graduate Studies

May 30, 2013

_____

Date

*Dedicated to my wonderful wife Jessica Breen Hays Lucas, and my sons Eli and Jonah, for (sometimes begrudgingly, but always lovingly) supporting and sustaining me through the course of this work.*

ACKNOWLEDGEMENTS

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**Introduction**

Since the 1950s modern agriculture has relied on mechanization and large amounts of external inputs including chemical fertilizers, herbicides, and pesticides to boost yields for a growing human population. While these advances have generally provided sufficient food for an increasingly growing population by overcoming soil fertility limitations and minimizing disease, pest and weed pressure, there have also been consequences to this model. Chemical fertilizer use has increased by 700% since the 1950's (Matson et al., 1997), leading to nutrient leaching or runoff and ultimately degradation of water quality (Matson et al., 1997). Heavy cultivation has led to soil degradation through erosion and loss of soil organic matter (SOM)(Lal, 1998) while monocropping has reduced biodiversity (Altieri, 1999). Because of these issues a growing number of scientists, policy makers, consumers, producers, and other stakeholders have raised concerns about modern agriculture and have expressed interest in developing a more sustainable agriculture (Matson et al., 1997).

Soil management to provide ecosystem services while simultaneously maintaining or enhancing soil quality is a key to sustainably managed agroecosystems (Lal, 2009). These ecosystem services are essentially "the benefits people obtain from ecosystems" (Millennium Ecosystem Assessment, 2005) and soil quality has been defined as "the capacity of a specific kind of soil to function, within natural or managed ecosystem

boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen, et al., 1997). Obviously there is overlap between these ideas and one could simplify the definition of soil quality to a soil's suitability to facilitate the provision of ecosystem services. In agroecosystems the chief ecosystem service is crop production.

Soil microorganisms play a major role in conserving or enhancing soil quality because so many soil processes flow through these organisms. Soil microbial communities are dynamic and rapidly adapt to environmental changes, including those caused by soil management (Kennedy et al., 2004). The soil microbial community, and the biodiversity of that community, has been identified as being important to soil quality, biodiversity and agroecosystem sustainability in general (Altieri, 1999; Brussard et al., 2007). Because so many ecological processes are controlled by the soil microbial community there is growing interest in manipulating or managing the soil microbial community for better provision of ecosystem services (Barrios, 2007).

**The Soil Microbial Community**

The soil microbial community is extremely diverse. One estimate, based on genomic DNA assessment, found an average of 10,000 different prokaryotic species per cm$^3$ of soil (Torsvik, et al., 2002). Soil microbial diversity is greater still when one considers eukaryotic members of the community, such as fungi. Straatsma et al. (2001) encountered over 400 fungal species through a 21 year study in soil from a Swiss forest while Giller et al. (1997) estimated there to be approximately 1,500,000 species of fungi, globally.

A soil's microbial community (along with its physical and chemical soil characteristics) plays a major role in the health and quality of that soil (Doran and Zeiss, 2000; Kennedy and Papendick, 1995). Among the most important of these processes is the decomposition of plant residues and other organic detritus (Kennedy and Papendick, 1995). Incorporated within this process is the formation of SOM and cycling of organic forms of N, P, and S. Soil organic matter plays a key role in soil quality (Weil and Magdoff, 2004) and availability of nutrients is critical to plant health. Beyond decomposition, microbes are important in other soil processes. They bolster availability of plant nutrients through mycorrhizal associations in plant roots and through chelation and solubilization processes. Some soil microbes fix nitrogen, a critical, often limiting, plant nutrient. Others promote plant growth and health through modulation of plant hormones, providing protection against phytopathogens, or enhancing tolerance to abiotic stresses such as drought and excess salt (Glick, 1995; Kloepper, et al., 1999; Yang, et al., 2009). Soil microbes also play a key role in building and stabilizing good soil structure, which in turn affects many soil functions associated with soil quality (Oades, 1993; Tisdall, 1991). The importance of these soil processes and the microbial roles in them cannot be understated. Price (1988) asserted that without microbial facilitation of soil processes, life as we know it would not exist.

Since microbes are the primary transformers of organic debris in soils, soil management practices such as the use of organic amendments can affect the activity (Sparling, 1997) and community structure (Schutter et al., 2001; Larkin et al., 2006; Saison et al., 2006; Buyer et al. 2010; ) of soil microorganisms. Further, microbial communities from specific management regimes such as organic production have been

shown to be different from those managed conventionally (Esperschütz et al., 2007).

Microbes within these dynamic communities can, in turn, have direct or indirect effects

on plant health and crop productivity.  An indirect microbial effect on plant health would

be microbial influence on soil structure, an important soil property that controls many soil

functional processes affecting crop health (Tisdall and Oades, 1982).  Direct effects

would include beneficial plant responses to soil microorganisms such as growth

promotion, improved disease resistance, or enhanced nutrient assimilation.


**Soil Aggregation: Interaction Between Soil Organic Matter and Soil Microoganisms**

Soil aggregates are the building blocks for soil structure in surface horizons.  By

governing processes such as water infiltration and movement (Prove et al. 1990), oxygen

diffusion (Sexstone et al., 1985), and plant nutrient availability (Six et al., 1998),

aggregate dynamics are strongly related to soil quality in agroecosystems.  Well

aggregated soils sequester soil organic matter (SOM) by physical protection of materials

in intra-aggregate spaces (Jastrow and Miller, 1998).  Soil surface crusting (Pagliai and

Antisari, 1993), surface run-off, and erosion are also mitigated in soils having good

structure (Bronick and Lal, 2005).  Plant root systems develop better, penetrate deeper,

and have access to more readily available nutrients in well aggregated soils (Lal, 1991).

According to Martin et al. (1955) aggregates are clusters of soil particles where

"the forces holding the particles together are much stronger than forces between adjacent

aggregates".  By the early 1900s the primary factors involved in soil aggregation

processes: soil fauna, microorganisms, plant roots, inorganic binding agents, and

environmental factors were known to soil scientists (Six et al., 2004).  Building on

previous research by Emerson (1959); Harris et al. (1966) and Edwards and Bremner (1967), Tisdall and Oades (1982) proposed the hierarchical model for soil aggregation. This model has become the most widely accepted soil aggregation model. It incorporates the primary factors mentioned above to describe the interaction of three types of physical units in soils: free primary particles, microaggregates, and macroaggregates (>0.25mm). In this model, soil microorganisms and SOM play major roles in formation and stabilization of aggregates. Aggregate formation and stabilization involves transient processes such as binding through microbial polymeric exudates (Haynes et al., 1991), temporary processes such as enmeshment in roots and fungal hyphae (Beare et al., 1997); and persistent binding through clay-organic matter complexes (Tisdall and Oades, 1982). Aggregation is also a function of the interaction of environmental factors, soil management factors, vegetation, and inherent soil properties such as parent material, texture, and exchangeable cations (Kay, 1998). Oades (1993) concluded that microbial factors become more important in aggregate formation when soils have little (<15%) clay.

In the Tisdall and Oades (1982) model aggregation occurs in stages where microaggregates form when primary particles are persistently bound by humic substances and polyvalent metal cation complexes. Microbial and plant polysaccharides and enmeshment by fungal hyphae or plant roots can subsequently bind microaggregates to one another, forming macroaggregates. Macroaggregates also form when labile particulate organic matter (POM) is deposited in soil, stimulating high microbial activity. Soil POM is defined as all SOM particles between 53 μm and 2 mm in size (Cambardella and Elliot, 1992). Microbial polysaccharides bind surrounding soil materials together

around POM (Jastrow, 1996) and as POM decomposes, microaggregates form within the macroaggregate (Beare et al., 1994).  As POM is exhausted and microbial activity declines, fewer binding agents are produced, resulting in reduced macroaggregate stability.  These macroaggregates ultimately break down, releasing highly stable microaggregates (Angers et al., 1997).

Soil aggregate dynamics are closely linked with SOM and soil carbon dynamics. Management practices that build SOM also tend to enhance aggregation while management that depletes SOM tends to reduce aggregate stability (Bronick and Lal, 2005).  For example, tillage and monocropping have been demonstrated to be deleterious to both soil aggregate stability and SOM content of a soil.  Organic amendments such as manure additions, compost additions, and cover crops (Bronick and Lal, 2005) have been observed to positively affect SOM and aggregation.

Soil microbes interact with soil organic matter such as fresh amendments and soil particles during aggregation processes.  Lynch and Bragg (1985) observed that organic matter additions to soils did not stimulate aggregation unless microbes were present. Some researchers found correlations between microbial biomass and aggregate stability (Drury et al., 1991) but others found no relationship (Carter et al., 1994).  Under drought conditions, Chantigny et al. (1997) saw a reduction in microbial biomass, at the same time aggregate stability increased.  The work by Drury et al., (1991) and that of Chantigny et al. (1997) suggests that the activity of specific microbial groups is more important to aggregate dynamics than the size of the microbial biomass.  These studies suggest that fungi, in particular, are key players in aggregate formation and stability.

Fungi are known to be very important in formation of macroaggregates. Studies have shown a decline in macroaggregate stability when antifungal compounds were used to inhibit fungal activity (Beare et al., 1997; Bossuyt et al., 2001). Because of the size of fungi, relative to that of bacteria, fungi influence aggregation at a macroscopic scale. Fungal hyphae bind macroaggregates together by physically enmeshing microaggregates and soil particles (Tisdall and Oades, 1982; Tisdall, 1991; Beare et al., 1997). The extracellular polysaccharides produced by fungi also act as aggregate binding agents (Chenu, 1989; Tisdall, 1991). Because of their much smaller size, bacteria are able to live in micropore spaces where they are protected from size-excluded bactivores (Heijnen et al., 1991). In these micropores, bacteria affect aggregation on a smaller spatial scale than fungi by excreting polysaccharides which bind silt and clay particles together (Lynch and Bragg, 1985) ultimately forming microaggregates (Oades, 1993). Researchers have observed higher fungi to bacteria ratios in macroaggregates relative to microaggregates (Gupta and Germida, 1988).

While it is known that aggregation processes (Martens, 2000; Abiven et al., 2007) and microbial community dynamics (Bossuyt et al., 2001; Bending et al., 2002) are affected by the type, quantity and biochemical composition of amendments used, a gap in the current literature exists with respect to applying this information. The effects of different soil amendments, relative to each other, on bacterial and fungal dynamics and associated effects on aggregate formation and stabilization have not been extensively investigated. Information from studies that compare the effects of different, commonly used amendments on microbial community dynamics and associated effects on soil aggregates would be useful to researchers and producers interested in maximizing the

agronomic benefits associated with maintenance of good soil structure.  Understanding which management practices stimulate fungi-facilitated structural enhancement would be particularly useful for strategizing soil amendment plans on farms.


**Soil Microbial-Plant Interactions to Promote Plant Health**

Microbial promotion of soil aggregation would indirectly facilitate production of healthy crops through enhanced soil functioning associated with good soil structure.  An important concept within the modern sustainability movement is that soil microbial biodiversity in agroecosystems can also directly benefit crop plants (Brussard et al., 2007).  Interest in the plant growth promoting potential of soil microbes (aside from symbionts such as mycorrhizhae or the nitrogen-fixing bacteria associated with legumes) began in earnest in the 1960s when scientists from the Soviet Union recognized the "biofertilization" potential of certain strains of *Azotobacter* sp. and *Bacillus* sp. (Mishustin and Naumova, 1962).  Since then several reviews have documented the, now extensive, work being done to understand and apply these plant growth promoting rhizobacteria (PGPR) (Glick, 1995, Gray and Smith, 2005).   The applied research has mainly focused on developing inoculants that suppress disease or promote growth (Glick et al., 1999; Sturz and Christie, 2003).  A major hurdle with these approaches is that these PGPR numbers often dwindle after introduction because they cannot compete for resources with indigenous flora (Cummings, 2009).

Recently Kumar et al., (2004) found that soil management may affect plant genetic responses and augment plant systems biology.   Kumar et al. (2004) observed that tomatoes (*Solanum lycopersicum* L.) grown after hairy vetch (*Vicia villosa* Roth) showed

modulation of a highly specialized, specific network of genes, relative to tomatoes grown with black plastic mulch and inorganic N fertilizer. Among the genes affected are some that delay leaf senescence including *rbcS, rbcL* (which code for the small and large subunits of RUBISCO, respectively). In tomatoes that followed hairy vetch, these genes were expressed at higher levels, later in the plant's life, compared to tomatoes grown with black plastic. Kumar et al., (2004) also found differences in genes involved in C/N signaling and plant defense in tomatoes grown after hairy vetch. These genes included *GS1* (which codes for a glutamine synthetase), *NiR* (which codes for nitrite reductase), *ChiB* (which codes for a defense related chitinase enzyme), *Osm* (osmotin: another defense protein), plastidic *G6PD* (plastidic glucose 6-phosphate dehydrogenase) and *SAG12* (senescence associated gene 12). They found GS1, *NiR*, *chiB*, osmotin, and plastidic *G6PD* to accumulate and persist longer in tomatoes grown after hairy vetch, relative to tomatoes grown in black plastic. However they found that *SAG12* transcript levels were higher in black plastic grown tomatoes. The *SAG12* gene codes for an enzyme that is involved in the cellular disassembly processes that lead to senescence (Lohman et al., 1994). The study by Kumar et al., (2004) was carried out in field grown tomatoes; however, in a related study Kumar et al. (2005) found similar results in greenhouse grown tomato plants. The results of Kumar et al. (2004) and Kumar et al. (2005) suggest that overall plant health is improved in tomato plants that follow hairy vetch cover crops. Kumar et al. (2004) described their findings as a "distinct expression profile" associated with the alternative agricultural practice of cover cropping with hairy vetch.

More recent research has also demonstrated that soil management can affect gene expression in plants. Lu et al. (2005) observed differential expression of nitrogen response genes between wheat (*Triticum aestivum* L.) grown in manure fertilized soil and that from soil fertilized with inorganic ammonium nitrate fertilizer. Kavroulakis et al. (2006) found that application of a disease suppressive compost increased expression of pathogenesis-related *PR* genes, possibly enhancing plant resistance to pathogen attack. Tenea et al. (2012) found that wheat grown with organic production practices had differential expression of ten signature transcripts when compared to wheat grown with conventional management.

Matoo and Abdul-Baki (2006) expand upon the work of Kumar et al. (2004) in a recent review of crop genetic responses to management practices. They note that it is highly probable that soil microbes play a role in eliciting favorable genetic responses in plants. They suggest that cytokinin producing soil microbes may play a role in the effect seen in the tomatoes of Kumar et al. (2004). These suggestions should be investigated given that others have demonstrated that some plant growth promoting organisms influence gene expression in plants (Park and Kloepper, 2000; Bent, 2006). The literature is sparse with regards to the influence of the indigenous soil microbial community on modulation of plant health related genes. Further, despite the competition hurdles seen with PGPR applications, little has been done to attempt to manage soil microbial communities *in situ* such that the population of indigenous organisms directly bolsters plant health through plant gene modulation.

**Managing the Soil Microbial Community with Organic Amendments**

Organic amendments are typically used to bolster the nutrient content of a soil to help supply the needs of crops. Cover crops such as the legume hairy vetch affect nutrient cycling through N additions by nitrogen fixation (Frye et al, 1988). Other winter cover crops such as cereal rye (*Secale cereal* L.) take up residual N, part of which becomes mineralizable N in SOM as the cover crop decomposes when killed in spring (Seiter and Horwath, 2004). Since around 2000 B.C. animal manures have been an important nutrient source in agriculture and were a primary nutrient in most systems before use of chemical fertilizers became widespread (Parr and Hornick, 1992). Likewise, from as far back as ancient Greek civilization, people have been composting organic substances and using the end product as a soil nutrient source and soil conditioner (Rodale, 1960).

Organic amendments build SOM (Seiter and Horwath, 2004), a critical component of soil quality in sustainable agrosystems (Weil and Magdoff, 2004). Because tillage generally leads to a decline in SOM levels, the use of organic inputs to build SOM is particularly important in systems where tillage plays a role in seedbed preparation and weed suppression. In a three-year study in conventionally tilled soils with high C mineralization rates, Sainju et al. (2000) found that the use of rye cover crops maintained total SOM levels in soils. Marriot and Wander (2006) saw SOM increases in organically managed soils amended with legumes or manure and noted that these increases occurred despite tillage.

One reason that the SOM building capacity of organic amendments is important is because a soil's SOM content strongly influences soil biology. The labile fractions of

SOM serve as the primary carbon substrate for the microbial flora and fauna found in a particular soil ecosystem (Weil and Magdoff, 2004) thus it should not be surprising that the microbial biomass in a soil is generally a reflection of total SOM content of the soil (Sparling, 1997).

It is not simply the SOM amount that drives soil microbial ecology. The amount, type, and quality of input can impact the soil microbial community. For example, the lability or complexity of amendments can affect the microbial community. Schutter and Dick (2001) used cellulose vs. simpler substrates such as glucose or gelatin and found fungal biomarkers to increase in the presence of the more complex cellulose. Plant inputs can affect soil microbial community dynamics differently than manure or compost inputs and the C:N ratio of inputs may affect community dynamics as well. Larkin et al. (2006) observed that manure inputs increased bacterial populations, particularly Gram negatives, while having inconclusive results on the fungal community. Carrera et al. (2007) found that fungal biomarkers were increased in soil following a vetch cover crop. Schutter et al. (2001) also observed increased fungal biomarkers with cover cropping. Wander et al. (1995) found that cover crops fostered high microbial biodiversity while manure amended soils had a more homogenous community but greater metabolic activity. Larkin et al. (2011) saw increases in mycorrhizal biomarkers in compost amended soils, while Saison et al. (2006) saw increases in bacterial and fungal biomarkers with compost.

**Can the Microbial Community be Managed to Promote Beneficial Agronomic Outcomes?**

While the understanding of soil microbial interactions with plants and the environment remains somewhat of a "black box" (Cortois and De Deyn, 2012), there is a substantial push among researchers to shine a light into said box, particularly with respect to managing what lies inside the box.  In reviewing effects of soil microbial diversity on crop health, Alabouvette et al. (2004) state that "much more research is needed to clearly understand the effects of management practices on diverse components of soil health." Brussaard et al. (2007) also cite the need for further research to optimize the use of organic amendments for stimulating soil biodiversity in different agroecosystems. Barrios (2007) identified six research priorities pertinent to understanding how the functioning and diversity of the soil microbial community is related to ecosystem services and soil productivity.  Among these priorities is the idea that scientists should use "understanding about hierarchical relationships to manage soil biota and function in cropping systems".  The results of others, when reviewed in conglomerate, suggest that soil microbial communities can be intentionally influenced with strategic application of amendments or management practices.  Jastrow et al. (2007), noting the relationship between SOM sequestration and soil structure, suggested just such a management scheme when they implied that one option for SOM stabilization is to modify the soil physicochemical environment such that fungal growth is promoted.   Much value is placed on managing soil microbial diversity in sustainable agroecosystems, however little information is available that provides understanding of how this management would directly or indirectly influence crop health.

The goal of this dissertation research was to begin filling this information gap by asking two research questions:  1) *Can soil management using organic amendments be used to develop a more fungi dominated community that ultimately fosters improved soil structure*?  2)  *Do various soil management practices have an effect on health-related gene expression (namely nitrogen assimilation gene expression and defense response gene expression) in plants; and if so, are there relationships between differential gene expression and management-influenced changes in the soil microbial community?* Providing answers to these questions would add to the understanding of soil management impacts on soil microbial ecology, soil quality, and physio-genetic responses in plants. This information would also facilitate the development of management strategies in production systems, such as organic production, that make heavy use of organic amendments.

**CHAPTER 2**

**IMPROVING SOIL STRUCTURE BY PROMOTIONG FUNGAL ABUNDANCE
WITH ORGANIC SOIL AMENDMENTS**


**Introduction**

Soil aggregates are the foundation for A horizon soil structure, and aggregate

dynamics influence how well a soil functions for crop production.  Generally, in well

aggregated soils, water availability, movement, and infiltration are enhanced while

surface crusting, run-off, and erosion are reduced (Bronick and Lal, 2005).  Soil

aggregation also affects oxygen diffusion, plant nutrient availability, development of

plant root systems, and soil organic matter (SOM) dynamics (Lal, 1991; Bronick and Lal,

2005).  Modern agriculture has historically degraded structure in many soils, leading to

deterioration of soil quality through erosion and losses of SOM, thus managing soils with

structure in mind has become important to those interested in sustainable agroecosystem

management (Lal, 1991).

Aggregation is a reorganization of primary soil particles into clusters where,

according to Martin et al. (1955) "the forces holding the particles together are much

stronger than forces between adjacent aggregates".  The environmental, biological, and

chemical factors that mediate soil aggregation have been thoroughly reviewed in the

literature (Kay, 1998; Bronick and Lal, 2005) and include available water, texture, parent

material, exchangeable ions and nutrients, SOM content, and the microbial community.

Of these factors, SOM and the microbial community are the most readily manipulated

through soil management to produce lasting improvements in soil structure (Bronick and

Lal, 2005).  Soil and crop management practices that increase SOM tend to improve

aggregation while those that reduce SOM tend to degrade soil structure. Excess tillage and monocropping can degrade aggregate structure while additions of organic amendments such as manure, compost, and cover crops have been shown to have a positive effect on SOM and aggregation (Bronick and Lal, 2005).

Tisdall and Oades (1982) proposed a hierarchical framework for aggregate formation and stability in which soil microorganisms and SOM play major roles in binding aggregates consisting of three types of physical units: free primary particles, microaggregates, and macroaggregates (>0.25mm). In this model, which was largely corroborated by the research of Elliot (1986), microaggregates form when primary particles are persistently bound through clay-SOM complexes. Microaggregates and primary particles are bound to form macroaggregates via the "glue" of microbial and plant polysaccharides, along with enmeshment by fungal hyphae and plant roots. These macroaggregates are particularly sensitive to changes in management, showing rapid (< 2 years) responses to management practices such as crop rotation (Haynes et al, 1991) and tillage (Chan et al., 2002).

Microbially mediated macroaggregate formation is promoted in soils by the presence of particulate organic matter that stimulates microbial activity (Jastrow, 1996). In several studies, addition of labile carbon substrates to soil induced rapid increases in microbial activity and concurrent increases in aggregate stability (Martens, 2000; Abiven et al. 2007). Lynch and Bragg (1985) observed that organic matter additions to soils did not stimulate aggregation when microbes were inhibited. The relationship between aggregation and microbial activity is more complex than sheer microbial numbers. Some researchers found positive correlations between microbial biomass and aggregate stability

(Drury et al., 1991), while others found no relationship (Carter et al., 1994) or a negative relationship (Chantigny et al., 1997). These findings suggest that aggregate dynamics might be related to the activities of specific microbial groups within the community. Drury et al. (1991) suggested that the reed canarygrass (*Phalaris arundinacea* L.) studied in their experiment promoted fungal activity, resulting in improved aggregate stability. Chantigny et al., (1997) saw that increases in glucosamine (an amino sugar that has been used as a fungal biomarker in soils) coincided with improved aggregate stability and concluded that fungi are likely the most important members of the soil microbial community with regards to impact on aggregation.

The mechanisms by which fungi bind macroaggregates have been described in the literature (Gupta and Germida, 1988; Tisdall, 1991; Beare et al., 1997). Studies have shown reduced macroaggregate stability when fungi were inhibited with fungicides (Beare et al., 1997; Bossuyt et al., 2001). Bacteria can live and facilitate aggregate binding in micropore spaces where other organisms are size-excluded (Heijnen et al., 1991). In contrast, fungi operate on a more macroscopic scale, binding aggregates by surrounding soil particles and microaggregates with hyphae (Tisdall, 1991). Evidence for these differences in scale is seen in studies where researchers have observed different proportions of fungi and bacteria in different aggregate size classes, with macroaggregates having greater fungi to bacteria ratios than microaggregates (Gupta and Germida, 1988). Given the importance of fungi in aggregate formation, information on agricultural management practices that increase fungal presence in a soil agroecosystem may be useful to producers who need to build soil structure.

Various amendments affect the soil microbial community in different ways, depending on amendment C:N ratio, biochemical composition, and complexity of available carbon substrates. Schutter and Dick (2001) analyzed ester-linked fatty acid methyl ester (FAME) biomarkers and found that soils amended with cellulose had elevated levels of fungal biomarkers relative to soils amended with simpler substrates such as glucose or gelatin. Such differences have also been seen in amendments commonly applied to soils. Larkin et al. (2006) found that manure inputs caused increased bacterial populations, while Carrera et al. (2007) found vetch cover crops to increase fungal phospholipid fatty acid biomarkers. Schutter et al. (2001) also saw increased fungal biomarkers in soils following cover crops. Wander et al. (1995) found cover crops to foster the greatest microbial diversity while manure amended soils were less diverse but had a more metabolically active biomass. Both Larkin et al. (2011) and Saison et al. (2006) observed that compost additions increased fungal biomass.

Jastrow et al. (2007) suggested that soil management practices could be used to improve soil structure (and ultimately sequestration of soil C) by altering the soil physicochemical environment such that fungal growth is promoted. Some researchers have examined the effects of plant residues on fungi and aggregate formation in soils where native structure has been destroyed. De Gryze et al. (2005) observed that aggregate formation increased with increasing amounts of wheat residue and, in two of three soils studied, also corresponded with fungal hyphae production. Helfrich et al. (2008) observed maize residues to rapidly stimulate increased macroaggregate formation; however, macroaggregate formation was delayed when a fungicide was also applied to the soil. While these studies build on the concept that management practices might be

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used to drive fungal mediated aggregate formation, there is a lack of studies that simultaneously compare multiple, commonly used amendments, such as vetch, manure, and compost, across different soils, for effects on the microbial community and corresponding changes in soil aggregation.

This experiment was designed to simultaneously compare the efficacy of hairy vetch, dairy manure, and compost, at promoting fungal proliferation and stimulating macroaggregate formation.  It was hypothesized that amendments that stimulate greater fungal presence in a soil will also lead to greater formation of stable macroaggregates. Information on which amendments enhance fungal biomass and stimulate macroaggregate formation, and information on how these amendments perform relative to each other, would fill knowledge gaps for researchers, extension agents, and producers interested in developing management strategies to maintain or enhance soil structure.


**Materials and methods**

*Soil Collection and Processing*

Three agricultural soils with different textures and chemical properties were used in this study: Maury silt loam (Fine, mixed, active, mesic Typic Paleudalfs; 11% sand, 74% silt, 15 % clay; CEC: 15.5 $cmol_c$ $kg^{-1}$; 76 % base saturation); Salvisa silty clay loam (Fine, mixed, active, mesic Mollic Hapludalfs; 19 % sand, 54 % silt, 27 % clay; CEC: 22.9 $cmol_c$ $kg^{-1}$; 68 % base saturation); and Yeager sandy loam (Sandy, mixed, mesic Typic Udifluvents; 76 % sand, 17 % silt, 7 % clay; CEC: 9.2 $cmol_c$ $kg^{-1}$; 15 % base saturation).  Other basic soil chemical properties are presented in Table 2.2.  In autumn of 2009, soils were collected to a depth of 15 cm, passed through a 4 mm sieve, and air

dried at 4°C.  All soil characterization was conducted by the Division of Regulatory

Services at the University of Kentucky.  Methods used are given in Soil and Plant

Analysis Council (2000), unless noted otherwise.  Briefly, soil pH was determined in a

1:1 soil:water paste using a calibrated pH meter and electrode, CEC and base saturation

via the ammonium saturation method, and Mehlich III extractable elements (P, K, Ca,

Mg, Zn, Na, Fe, and Al) were quantified by inductively coupled plasma

spectrophotometry.  Total organic C (TOC) and total N (TN) were quantified via a LECO

dry combustion instrument and particle size analysis by the micropipette method of

Miller and Miller (1987).


*Soil Incubation Experiment*

Before preparing soil treatments, the native structure of soils was destroyed by

forcing through a 250 µm sieve, and then mixing back the sand sized particles that were

removed with the 2000 µm sieve.  Then, four treatments were imposed on each soil,

including 1) no amendment (control), 2) hairy vetch (*Vicia villosa* Roth), 3) dairy

manure, and 4) vegetable compost. The vetch was a winter cover crop planted in fall

2008 at The University of Kentucky Horticulture Research Farm in Lexington, KY and

harvested in spring 2009.  Fresh dairy manure was obtained from the University of

Kentucky Dairy Research Facility, Lexington, KY.  The green-waste based compost was

purchased from Peaceful Valley Organic Supplies (Grass Valley, CA).  Compost was air-

dried for 48 hours, and the vetch and manure were dried at 65°C and subsequently

allowed to equilibrate to air-dry moisture content before being used in experiments.  All

amendments were forced through a 2 mm sieve.

Amendment total C and total N were characterized using LECO dry combustion. A proximate organic C distribution of amendments was determined using a fractionation procedure that segregates total organic carbon into lipid C, water soluble C, acid soluble C, and lignin and humic C pools (Ryan et al., 1990; D'Angelo et al., 2005). Elemental analysis (Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, P, Ni, and Zn) of organic amendments was determined using the nitric acid digestion procedure described in D'Angelo et al. (2012). Digests were analyzed for metals and metalloids using a Varian Vista-PRO Inductively Coupled Argon Plasma (ICP) (Palo Alto, CA) by the University of Kentucky, Division of Regulatory Services. Amendment characteristics are presented in Table 2.1.

The various soil-amendment treatments were prepared by thoroughly mixing in one of the three amendments at a rate of 0.01 g amendment C $g^{-1}$ soil. This was equivalent to 89, 26, and 25 mg amendment $g^{-1}$ soil for compost, vetch, and manure respectively. For each of the twelve soil-amendment combinations, fifteen microcosms were prepared (three for each of the five time steps), which consisted of 473 mL wide-mouth glass canning jar with a 70 mm glass microfiber filter (Whatman GF/C) placed at the bottom. An aluminum ring having a 6.0 cm height and 5.3 cm internal diameter was placed on top of the filter. Approximately 72 g of the treated soil was placed into the aluminum ring, and sufficient water was added to the outside of the ring to bring the soil to field capacity by capillary movement of water from the filter to the soil. Constant soil moisture content was maintained during the incubation by periodic addition of the appropriate amounts of water to maintain constant microcosm mass. Microcosms were incubated at 25°C for 82 d, and destructively sampled on days 0, 5, 12, 30, 82 for microbial community and aggregate size distribution analysis. Subsamples for aggregate

size distribution were stored at 4°C for up to 4 days before analysis, and subsamples for microbial community properties were stored at -20°C for up to 60 days before analysis, using the procedures described below.

*Size Distribution of Water Stable Aggregates*

Water stable aggregates were assessed via the wet sieving method of Elliot (1986), which yields four aggregate size classes: large macroaggregates (>2000 μm diameter), small macroaggregates (250-2000 μm), microaggregates (53-250 μm), and the silt + clay fraction (<53 μm).  Briefly, 25 g of treated soil was placed on a 2000 μm sieve. The sieve was submersed in a pan of deionized water to a depth of 3 cm above the sieve mesh for 5 minutes.  Starting with the 2 mm sieve, aggregates in the different size classes were obtained by collecting material that did not pass through the appropriately sized sieve after 50, 3-cm vertical motions of the sieve in the pan of water over a period of 2 minutes.  To obtain the next smaller sized aggregate fraction, the process was repeated using the material that passed through the previous sieve. Aggregate fractions that remained on sieves (or ultimately passed through final, 53 μm sieve) were oven dried at 65°C for 72 hours and weighed (± 0.01 g).  Aggregate fraction weights were used to calculate aggregate fraction size as a mass percentage of whole soil.

*Fatty Acid Methyl Ester Extraction and Quantification*

Ester-linked fatty acid methyl esters were extracted from soils as described in Schutter and Dick (2000).  Briefly, 3 g of soil was added to a 35 mL glass centrifuge tube, followed by  addition of 15 mL of 0.2 *M* KOH in methanol to release lipids from

microbial membranes.  Centrifuge tubes were sealed with Teflon-lined caps, mixed on a

vortex mixer for 20 s and incubated in a water bath at 37°C for 1 h.  At 10 min intervals,

samples were vortexed for 10 s to facilitate the release and methylation of FAMEs.  After

1 h, samples were neutralized with 1.0 $M$ acetic acid.  The FAMEs were partitioned into

an organic phase by adding 10 mL of HPLC grade hexane, vortexing for 60 s, and

centrifuging at 330     $g$ for 20 min.  Five mL of the hexane layer was transferred to a

clean, glass, screw-top test tube.  Methyl nonadecanoate was added as an internal

standard followed by evaporation of the hexane under a stream of $N_2$ gas.  Samples were

dissolved in 0.2 mL of a 1:1 mixture of hexane : methyl-tert butyl ether and analyzed

using a Shimadzu GC-14A gas chromatograph (Shimadzu, Columbia, MD) equipped

with a flame ionization detector and a Restek Rtx-1 column (100% dimethylpolysiloxane,

30 m by 0.25 μm, 0.32 mm ID) (Bellefonte, PA).  The temperature program ramped from

80°C to 260°C, increasing at 3°C per minute, followed by 10 min held at constant 260°C.

Fatty acid methyl esters were identified by comparing retention times against those from

the Supelco FAME mix C4-C24 (Supelco, Bellefonte, PA) and additional individual

FAME standards purchased from Matreya, LLC (Pleasant Gap, PA).

The fatty acid nomenclature used is described in Schutter and Dick (2000).  The

aliphatic (ω) end of the fatty acid served as the starting point for carbon numbering, and

the number after the colon represents the number of double bonds.  The suffixes "*c*" and

"*t*" denote *cis* and *trans* conformations, respectively, while the prefixes "*i*" and "*a*"

denote *iso-* and *anteiso-* branched fatty acids.  In addition, cyclopropane, and methyl

groups are denoted by "cy" and "Me" respectively.  Signature fatty acids were assigned

to specific soil microbial groups as described in the literature.  Groups examined included

fungi (linolenic acid, 18:2ω6c, Frostegård and Bååth, 1996; Zelles, 1999), bacteria (sum of i15:0, a15:0, 15:0, i16:0, 16:1ω7t, i17:0, a17:0, 17:0, cyl7:0, 18:1ω7c, and cyl9:0; Frostegård and Bååth, 1996), actinomycetes (sum of 10Me16:0, 10Me17:0, and 10Me18:0; Zelles, 1999), and arbuscular mycorrhizal fungi (16:1ω5c, Olsson, 1999). The relative abundance of each microbial group within the community was determined by dividing the amount of signature FAME by the total amount of FAME, and multiplying by 100 to convert to percent.

Although fatty acid methyl ester analysis is a useful tool for tracking changes in microbial groups in soils, one problem with the technique is nonspecificity of certain FAMEs. For example, 18:2ω6c is produced by both fungi and plants (Frostegård and Bååth, 1996). Therefore, in soils amended with vetch, it was expected that 18:2ω6c concentrations would initially be elevated due to the addition of vetch rather than growth of fungi. To account for this, ergosterol, which is a specific fungal biomarker, was measured in parallel with FAMEs. As expected, 18:2ω6c concentrations were not significantly correlated to ergosterol in vetch-amended soils on days 0 ($r = -0.25$), 5 ($r = 0.08$) or 12 ($r = 0.46$) of the incubation. However, 18:2ω6c and ergosterol were highly correlated on days 30 ($r = 0.88^{**}$) and 82 ($r = 0.90^{***}$) of the incubation, indicating that 18:2ω6c was largely of fungal origin at these times. These findings were consistent with work by Klamer and Bååth (1998) who observed rapid loss of plant derived 18:2ω6c during straw composting. On this basis, only FAME data collected from day 30 and day 82 is presented.

*Ergosterol Extraction and Quantification*

The fungal biomarker ergosterol was extracted from soils using the method described in Montgomery et al. (2000) as modified by Zhang et al. (2008). Modifications included shorter bursts of energy, shorter cooling times, and the addition of 2-propanol to the extractant mixture. In brief, 0.25 g of soil was added to 35 ml glass centrifuge tubes, followed by the addition of 2 mL of methanol, 2 mL of 2-propanol, and 1 mL of 2 *M* NaOH. Tube threads were wrapped with Teflon tape and tightly capped with Teflon-lined caps. Each sealed tube was placed into a separate 500 mL high density polyethylene bottle, which was tightly capped and placed into the center of a Sanyo EM3320S microwave oven (Sanyo Corporation, Moriguchi, Osaka prefecture, Japan). Samples were irradiated individually for 10 s at 2450 MHz, 525 W output and then allowed to cool to room temperature for 3 min. The irradiation-cooling process was repeated four times (total of 40 s of irradiation). Samples were then neutralized using 2 mL of 1 *M* HCl. Ergosterol was partitioned to an organic phase by adding 2 mL of distilled water, 3 mL of pentane, and vortexing for 30 s. To separate phases, samples were centrifuged at 330 *g* for 10 min. The pentane (upper) phase containing ergosterol was transferred to a clean glass test tube with a Teflon-lined screw-top. This extraction and transfer process was repeated two more times (for a total of 9 mL pentane) and collected into the same tube. The pentane was filtered with a Teflon 0.45 μm syringe filter (National Scientific, Rockwood, TN) to remove particulates, and then evaporated to dryness under a gentle stream of $N_2$ gas. Ergosterol was redissolved in 0.2 mL methanol and analyzed on a Shimadzu LC-10A HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-10AD pump, a SIL-10AD automatic sample injector, a DGU-14A degasser, a

CTO-10A column oven, a SPD-10A UV-Vis detector, and a SCL-10A system controller. Separations were carried out on a Phenomenex Synergi 4u Hydro-RP 80A reverse phase C-18 column (4.6 x 150 mm) (Torrance, CA) using a methanol mobile phase at a flow rate of 1.3 mL min$^{-1}$. The SPD-10A detector was set to a wavelength of 282 nm. Ergosterol concentrations in samples were determined by comparing sample peak areas to a standard curve generated with six external standards ranging from 0.5 µg mL$^{-1}$ to 50 µg mL$^{-1}$ that were prepared from a standard obtained from MP Biomedicals (Solon, OH).

*Statistics*

The experiment was conducted using a randomized complete block design with three replicates of each soil-treatment combination for each sampling date. Treatment effects on individual microbial groups and aggregate fractions were analyzed by ANOVA and post-hoc Fisher's LSD means separation test. Simple Pearson's correlations were used to examine relationships between LMA and selected microbial biomarkers. These analyses were carried out using SYSTAT version 13 (SYSTAT Software, 2009).

The multi-response permutation procedure (MRPP) was used to determine whether microbial community composition was significantly different in the four treatments. Non-metric multidimensional scaling (NMS) was used to determine the interrelationships between the organic amendment treatments, formation of aggregates, and microbial community biomarkers, which were illustrated in joint-plots. MRPP and NMS analyses were carried out using PC-Ord version 5.1 (MJM Software, 2006).

**Results**

*Effects of Organic Amendments on Soil Properties*

The addition of organic amendments had significant effects on several chemical properties of the soils (Table 2.2). Amendments significantly increased total organic carbon in soils by 1% and increased total nitrogen by about 0.1% in all the soils. The amendments also significantly increased Mehlich III P and K in the soils. Compost and manure significantly increased Mehlich III Ca, Mg, and Na, while vetch increased Mehlich III Mn in the soils. Manure significantly increased pH of the Salvisa and Yeager soils, but not the Maury soil. Depending on the treatment, pH decreased by 0.4-0.8 units in Maury, 0.6-1.2 units in Salvisa, and 0.8-2.2 units in Yeager, after 82 d of incubation (Table 2.2). After 82 d of incubation the total organic carbon content in the manure and vetch treatments was significantly reduced in all soils. Total organic carbon was also significantly reduced in the Salvisa control soil over the course of the incubation. Significant reductions did not occur in any compost amended soils or in the control treatments for the Maury and Yeager soils. All other chemical parameters were relatively constant during the incubation.

*Effects of Organic Amendments on Formation of Large Macroaggregates.*

Organic amendments had significant effects on formation of LMA in all of the soils (Figure 2.1). In the manure and vetch treatments, effects were evident as early as 5 d after the start of the incubation. Vetch stimulated the greatest LMA formation in all soils; within 5 d after amendment, LMA increased from 0% to 45%, 63% and 81% of the whole soil mass in the vetch amended Yeager, Maury, and Salvisa soils, respectively and

remained elevated during the remainder of the experiment. The addition of manure to the Salvisa soil also increased LMA formation relative to the control within 5 d of the incubation. The addition of compost either had no effect on LMA formation (Salvisa), or reduced LMA formation (Maury and Yeager) relative to the control during the incubation.

*Effects of Organic Amendments on Soil Microbial Communities*

Amendment treatments had significant effects on the microbial community composition and structure. By day 30, interferences (see section 2.4) with plant derived FAMEs in vetch-amended soils were negligible and amendment treatments had significant effects on concentrations of microbial FAME biomarkers in soils (Table 2.3). Total FAME concentrations in the control soils on day 30 ranged between 172 and 302 $\mu$mol kg$^{-1}$ soil, and was highest in the Maury soil compared to Salvisa and Yeager soils. Relative to the control soils, amendments significantly increased total FAME concentrations by 82 to 491% (average 233%); vetch or manure increased total FAME more than compost. By the end of the incubation, total FAMEs in the treatments were reduced from day 30 levels by up to 34%, but were still higher than in the control soils.

Bacterial FAME concentrations in the control soils at day 30 ranged between 46 and 80 $\mu$mol kg$^{-1}$ soil, and were highest in the Maury soil compared to Salvisa and Yeager soils (Table 2.3). Amendments significantly increased bacterial FAMEs by 68 to 219% (average 151%) relative to the control soils; manure and vetch increased bacterial FAMEs more than compost. By day 82, bacterial FAMEs in the treatments were reduced from day 30 values by up to 41%, but were still higher than in control soils. In terms of

relative abundance, bacterial FAMEs made up between 15 and 32% (average 24%) of total FAMEs in the two time periods; vetch-amended Salvisa and Yeager soils had significantly lower bacterial FAME abundances than other treatments.

Fungal FAME concentrations in the control soils at day 30 ranged between 6 and 32 µmol kg$^{-1}$ soil, and were highest in the Maury soil compared to Salvisa and Yeager soils (Table 2.3). Amendments significantly increased fungal FAMEs by 22 to 2800% (average 920%) relative to the control soils; manure and vetch increased fungal FAMEs more than compost, which was particularly evident in the Salvisa and Yeager soils. From day 30 until day 82, fungal FAMEs in most of the amended soils were reduced by up to 44%, except in the manure-amended Yeager soil in which this group increased by about 2%. The relative abundance of fungal FAMEs during the incubation ranged between 4 and 31% (average 13%), and was significantly higher in the vetch- and manure-amended soils.

Actinomycete FAME concentrations in the control soils at day 30 ranged between 8 and 13 µmol kg$^{-1}$ soil, and was highest in the Maury soil compared to Salvisa and Yeager soils (Table 2.3). Amendments significantly increased actinomycete FAMEs by 14 to 147% (average 84%) relative to the control soils on day 30, with compost increasing levels to the greatest extent in all soils. Actinomycete FAMEs were relatively stable during the incubation, making up between 1 and 6% (average 4%) of total FAMEs, with the highest relative abundances in the control soils compared to amended soils.

The concentration of the arbuscular mycorrhizal FAME (16:1ω5c) in the control soils at day 30 ranged between 4 and 9 µmol kg$^{-1}$ soil, and was highest in the Maury soil compared to Salvisa and Yeager soils (Table 2.3). Amendments significantly increased

arbuscular FAME by up to 760% (average 184%) relative to the control soils, with manure increasing levels by the greatest amount in the three soils. Arbuscular FAME was relatively stable during the incubation, making up between 1 and 6% (average 3%) of total FAMEs, with the highest abundances in the manure-amended Maury and Salvisa soils at the end of the incubation.

Ergosterol concentrations in the control soils at day 0 ranged between 0.9 and 2.6 mg kg$^{-1}$ soil, and were highest in the Maury, followed by Salvisa, and finally Yeager (Figure 2.2). Within 5 d, amendment of soils with vetch or manure led to rapid increases in ergosterol, which slowly declined during the remainder of the incubation. In general, ergosterol concentrations were highest in the vetch treatments and remained significantly greater than levels seen in other treatments throughout the course of the study. In contrast, ergosterol in the control or compost-amended soils did not change significantly during the incubation.

For both day 30 and day 82, the MRPP of the microbial group relative abundances revealed that treatments fostered significantly different microbial community compositions (day 30: $P<0.0001$, within group agreement = 0.48; day 82: $P<0.0001$, within group agreement = 0.41). Specifically, the relative abundance of bacteria was significantly lower, and the relative abundance of fungi was significantly higher in the vetch- and manure-amended soils compared to control or compost-amended soils (by 2-8 times).

The NMS ordination of FAME relative abundances for day 82 indicated that the microbial communities were different between treated soils. A Monte Carlo test of 500 runs with randomized data indicated the minimum stress of a two dimensional solution

was lower than would be expected by chance (p = 0.004). The final stress and instability of the two-dimensional solution was 6.74 and 0.00, respectively. The NMS ordinations of FAME relative abundance data for day 82 shown in Figures 2.3a and 2.3b show that 79% and 16% of the variation was explained by axis 1 and axis 2, respectively. The NMS analysis for day 30 FAME relative abundance was very similar (p = 0.004; stress = 8.19; instability = 0.00) with FAMEs showing a nearly identical ordination pattern and with axis 1 explaining 77% of the variation and axis 2 explaining 18%. On day 82, FAMEs that were strongly correlated to axis 1 included 18:2ω6 and 20:5ω3 (associated with fungi), and FAMEs that were negatively correlated to this axis included i15:0, i16:0, i17:0, a17:0, cy19:0 (bacteria), 10Me16:0 (actinomycetes), and 14:0, 16:0, 18:0, 20:4ω6, 20:0, 21:1, 22:0, and 24:0 (non-signature lipids) (Figure 2.3a). FAMEs that were strongly correlated to axis 2 included 18:3ω3 (attributed to fungi) and 18:1ω5 (bacteria), while i15:0, i17:1ω7, 18:1ω7 (all bacterial) and 16:1ω5 (arbuscular mycorrhizal fungi) had strong negative correlations with this axis. Similar relationships were seen in the NMS analysis of day 30 FAMEs (not shown).

**Discussion**

*Effects of Organic Amendments on Soil Chemical Properties*

Organic amendments used in the study (vetch, compost, and manure) had wide ranging chemical characteristics (Table 2.1) that significantly affected several soil important chemical properties (Table 2.2). All of the amendments significantly increased total organic C, total N, Mehlich III P, and K compared to control soils. Furthermore, compost and manure increased Mehlich III Ca, Mg, and Na. During the incubaton, pH

decreased in all treatments, likely due to microbial respiration, nitrification, and other oxidative processes under the aerobic conditions of the incubation. The greatest pH decreases were seen in the Yeager soil, followed by Salvisa, and Maury. Differences in pH changes between soils were likely attributable to variations in amounts of carbonates, clay minerals, and oxyhydroxides that buffered against pH changes.

Of the three amendments, the manure and vetch contributed the most labile and semi-labile C substrate to soils, as indicated by the proximal fractionation (Table 2.1). In this fractionation the most labile C resides in the water extractable C pool (sugars, amino acids, nucleotides, etc.) and semi-labile C is in the acid soluble C pool which is primarily comprised of cellulose (Ryan et al., 1990; D'Angelo et al., 2005). Lipids in the non-polar C pool are also semi-labile, while oils and waxes extracted in this pool are more resistant to decomposition, however this pool makes up a small portion of the total organic C in the amendments (Table 2.1). The humic and lignin C pool is very resistant to degradation (Stevenson, 1994). Only 24 % of compost C was in the labile water soluble and acid soluble C fractions, while manure and vetch, respectively, had 33 % and 54 % of their C in these pools, indicating that the latter amendments contributed more microbially available C. The greater microbially available C contributions of the manure and vetch treatments is evidenced by greater losses of organic C (about 20%) that occurred during the 82 d incubation in soils with these amendments (Table 2.2). In contrast, organic C losses were generally not observed in the control or compost-amended soils, indicating that organic C in these materials was relatively stable against biodegradation. The comparatively small (7%), but significant decrease in total organic C seen in the Salvisa control was likely the result of the release via sieving and subsequent degradation of

formerly protected C.  Such an effect is not surprising given that the Salvisa soil has a

greater clay content which should allow it to physically protect more C (Six et al., 2002)

than the other soils, allowing for greater potential C loss upon physical disruption.

Differences in organic C bioavailability of the amendments were not surprising

considering that they represented a spectrum of decay maturity, with compost

representing the most decayed, followed by manure, and finally vetch.


*Effects of Organic Amendments on Soil Microbial Communities*

Differences in organic C bioavailability and other chemical properties of the

amendments were expected to elicit major changes in both total microbial biomass and

distribution of microbial groups in the amended soils.  After amending soils, total

FAMEs increased considerably, particularly in soils amended with vetch, followed by

manure, and finally compost (Table 2.3).  This was likely a reflection of relative amounts

of microbially available organic C contributed by amendments.  This is supported by the

fact that the concentrations of total FAMEs were significantly and positively correlated ($r$

$= 0.62$; $p < 0.001$) with the amount of total C lost over the course of the incubation.

Other studies have also shown plant- and manure-amendments to increase microbial

biomass in soils (Frostegård et al., 1997; Peacock et al., 2001; Buyer et al., 2010).

The increased total FAME concentrations in the amended soils reflected increases

in the concentrations of bacterial, fungal, actinomycete, arbuscular mycorrhizal, and other

FAMEs.  The magnitudes that individual microbial groups were increased, however,

depended on the type of amendment (Table 2.3).  For example, vetch and manure

increased bacterial and fungal FAME concentrations more than compost.  This was

particularly evident in the Salvisa and Yeager soils which had low initial levels of these microbial groups. The effects of amendments on the fungal FAME biomarker were supported by results from the ergosterol analysis, which showed the greatest ergosterol concentrations in the vetch- and manure-amended soils compared to control and compost-amended soils during the incubation (Figure 2.2). Other studies found bacterial and fungal biomarkers were increased in vetch-amended soils (Buyer et al., 2010), manure-amended soils (Frostegård et al. 1997; Peacock et al., 2001), and compost-amended soils (Saison et al., 2006). However, similar to this study, Quintern et al. (2006) did not find any significant increase in ergosterol in soil amended with compost. Again, discrepancies are likely attributed to differences in the lability of C compounds within the amendments used in the studies.

Shifts in community structure are readily observed when FAME relative abundances are examined. As indicated by the MRPP analysis, the community compositions were significantly different between soil treatments with the relative abundance of fungi increasing in vetch and manure treated soils. This phenomenon can clearly be seen in the calculated fungal to bacterial ratios given in Table 2.3. In addition, within a few days after amending soils, fungal proliferation was visually obvious as a mycelial mat in vetch-amended soils. Microscopic examination of this mat revealed numerous septate mycelia along with a sporulating *Aspergillus* species. A likely explanation for fungal enrichment in the vetch- and manure-amended treatments was that they contained elevated levels of intact plant cell walls that fungi are well suited to utilize as substrates through production of extracellular cellulases, hemicellulases, lignin- and Mn-peroxidases (Carlile et al., 2001). This explanation is supported by the greater

amounts of acid soluble C observed in proximal fractionations of vetch and manure

(Table 2.1). Fungal growth in the vetch- and manure-amended soils could also have been

favored by higher available nitrogen (Rousk and Bååth, 2007) and lower pH (Rousk et

al., 2009). Several other studies have also observed fungal enrichment in soils amended

with vetch (Carerra et al., 2007; Buyer et al., 2010) and dairy manure (Peacock et al.,

2001; Larkin et al., 2006). On the other hand, Saison et al. (2006) and Larkin et al.

(2011) found that fungi were enriched by compost, which was not observed in this study.

A likely explanation for this discrepancy is that compost in various studies had different

biochemical makeup or different amounts were applied to soils. For example, the

compost used in this study was derived from vegetable matter and had a C:N ratio of

12:1, which is indicative of a mature compost. On the other hand, the compost used by

Saison et al. (2006) had a C:N ratio of 27:1, suggesting that it may have been less mature

and contained substrates that could be preferentially used by fungal groups. Evidence for

this explanation is seen in a study by Annabi et al. (2007) who found that fungal biomass

increased with immature composts but not with mature composts. In general, the results

of this study and other studies indicate that manure and vetch amendments tended to

enrich fungal biomass relative to that of bacteria, while compost did not have a similar

effect on microbial community structure due to its maturity and biochemical

recalcitrance.


*Effects of Organic Amendments on Formation of Large Macroaggregates*

It was hypothesized that amendments with different chemical characteristics and

organic C bioavailabilities would significantly affect aggregation development in a

variety of soils by a combination of chemical and biological processes. In this study, the formation of LMA was the primary focus because LMA dynamics have been shown to be sensitive to differences in soil management (Haynes et al, 1991; Chan et al., 2002) and LMA are critical in protecting soil organic matter from biodegradation (Six et al., 2000), reducing soil erosion, and improving water movement through the soil profile (Franzluebbers, 2002).

Amendment of soils with hairy vetch stimulated the greatest formation of LMA in all three soils, which was evident as early as 5 d after the start of the incubation (Figure 2.1). Others have also documented that vetch enhanced soil structure development (Sun et al., 1995; Sato et al., 2007). In contrast, manure and compost amendments did not have consistent effects on LMA formation in the soils. Although there was a general trend of increased LMA formation in manure-treated soils compared to control soils, the increases were only statistically significant in the Salvisa soil. This was likely explained by texture differences of the soils, which has been shown to be important in controlling macroaggregate formation in other manure-amended soils (Hafez et al., 1974). Also, it is likely that aggregate formation would have been observed after repeated manure applications, which has been shown in other studies (Martens and Frankenberger, 1992; Pare et al., 1999).

It was somewhat puzzling that compost was not effective at promoting LMA formation compared to the control soils in this study. These results were in contrast to other studies that showed improved soil structure development with compost amendments (de Leon-Gonzalez et al. 2000; Annabi et al., 2007; Tejada et al., 2009). One possible explanation is that the compost used in this study may have contained high

levels of monovalent cations compared to divalent cations, which could lead to dispersion of soil colloids via the large hydration sphere associated with monovalent cations. To explore this possibility, the concentrations of $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ presented in Table 2.2 were substituted into the Cations Ratio of Structural Stability (CROSS) and Sodium Adsorption Ratio (SAR) relationships described by Rengasamy and Marchuk (2011). Based on these analyses, clay particle dispersion was predicted to be <<1%, which indicated that other factors besides cation imbalances likely accounted for low macroaggregate formation in the compost-amended soils. Annabi et al. (2007) found that mature composts did not improve aggregate stability to the same degree as immature composts. Being comprised of 74% humic and lignin C (Table 2.1), the compost used in this study may have been too mature to promote macroaggregate formation.

A number of other factors could possibly account for differences in structure development in the various treatments, including variations in pH, Fe-, Mn- and Al-oxyhydroxides, types and amounts of clay minerals, cations, organic and inorganic carbon, as well as production of microbial exudates and hyphae (Bronick and Lal, 2005). To explore these possibilities, regression analysis was conducted between soil chemical properties and changes in LMA that occurred during the 82 d incubation. In all soils, the formation of LMA was significantly correlated to Mehlich III Mn (r=0.86-0.94, p<0.001), which suggested that amorphous Mn oxides may facilitate the formation of stable LMA in these soils by bridging mineral and organo-mineral particles. Alekseeva et al. (2009) also concluded that Mn oxides were critical in the formation of LMA across a range of soil types. Hairy vetch-amended soils contained elevated levels of Mn (Table 2.2), which may partially explain why these treatments had greater amounts of LMA.

Several other chemical properties were significantly related to aggregate formation (e.g. Mehlich K, Cu, Ca, and Fe); interpretation of these relationships, however, was difficult because data from the treatments tended to group at both ends of the regression line, which likely accounted for high significance levels between LMA formation and these parameters.

*Relationships Between Macroaggregate Formation and Microbial Community Structure*

It is well-known that bacteria and fungi in soils can play key roles in soil structure development by excreting polysaccharides, proteins, lipids, and other agents, as well as producing hyphae that can bind soil particles together (Tisdall, 1991). Thus, it was not surprising that LMA formation was highly correlated to total FAME concentrations in this study (r=0.57, p<0.001). Correlations between percentages of LMA and concentrations of individual FAMEs at day 82 were examined to determine the importance of various microbial groups in the formation of LMA. Based on this analysis, LMA formation was strongly correlated ($r \geq 0.55$, p<0.001) with concentrations of several biomarker FAMEs, including a15:0, 16:1ω7, cy17:0 and 18:1ω5, which represent bacteria (Zelles, 1999), and 18:2ω6, 18:3ω3, and 20:5ω3 which have been attributed to fungi (Olsson, 1999; Zelles, 1999). The importance of fungi was corroborated by the ergosterol results which indicated a strong correlation between this biomarker and LMA formation (r = 0.59, p < 0.001). In fact, a relationship between LMA formation and ergosterol (r = 0.82, p < 0.001) was observed as early as 5 days after the outset of the incubation, indicating that fungi play an important role in stabilizing soil structure shortly after carbon substrates are added to soils. In examining biomarker concentrations, it

appears that, in general, LMA formation is promoted when amendments stimulated proliferation of microbial groups.

To determine which microbial group(s) played more important roles in LMA formation, regression analysis was also conducted between LMA levels and the relative abundances of individual FAMEs in the various treatments at day 82. This analysis revealed that LMA formation was most strongly correlated ($r \geq 0.55$, $p<0.001$) to the abundances of three FAMEs attributed to fungi ($18:2\omega6$, $18:3\omega3$, and $20:5\omega3$) and negatively related to most bacterial FAMEs. These results indicate that fungi likely played larger roles than bacteria in facilitating LMA formation in the soils studied. They also may explain why LMA formation was greatest in the vetch treatment, which greatly stimulated the growth of fungi in all the soils.

Non-metric multidimensional scaling ordination was conducted with the relative abundances of FAME biomarkers and various soil properties in amendment-treated soils in order to further illustrate the relationships between microbial community composition, soil chemical and physical properties, and formation of LMA in the various treatments (Figure 2.3b). The NMS joint plot in Figure 2.3b clearly showed that on day 82 microbial communities were different in soils that received the different amendments. The corresponding joint plot for day 30 microbial communities was very similar to that of day 82 (data not shown). On day 82, vetch- and manure-amended soils were similar to each other, but they were different from the compost-amended and control soils, as shown by the relative positions of these treatments at the opposite ends of axis 1. The vector associated with soil K suggests that, relative to the other vetch-amended soils, the difference in ordination along axis 2 seen in the microbial community associated with the

vetch-amended Yeager was likely due to the K input from vetch residues (Table 2.1)

having a large influence on the microbial community in a sandy soil that normally has

low K levels (Table 2.2). Microbial communities associated with vetch and manure

treatments ordinated positively along axis 1 in the same direction as fungal-associated

FAMEs and ergosterol (Figure 2.3b). Also ordinating in this direction were the proxmial

amendment fractions associated with labile and semi-labile C (non-polar C, water soluble

C, and acid soluble C) as well as microbially available soil C as estimated by the loss of

total soil organic C from day 0 to day 82. Finally LMA formation also ordinated strongly

in the positive direction along axis 1, towards those microbial communities associated

with vetch and manure treatments, indicating a strong relationship and reinforcing

previously described importance of fungi in aggregate formation. These relationships

were similar in all soils. For example in NMS ordinations (not shown) conducted on

individual soils fungal FAME, amendment labile and semi-labile C parameters, and LMA

ordinated positively along axis 1 while bacterial FAMEs ordinated negatively. The NMS

ordinations strongly suggest that amendments containing high levels of microbially

available C fostered fungal proliferation in soils; increased fungal abundance was in turn

strongly related to LMA formation.

**Conclusions**

There were considerable differences in microbial community composition in a

range of soils that were amended with organic materials that contained variable amounts

of bioavailable organic C. Vetch and manure amendments contained higher amounts of

microbially available C which stimulated bacterial and fungal growth and formation of

LMA in the soils.    In contrast, compost contained the least microbially available C and did not stimulate fungal growth or LMA formation in any of the soils.   Future research should assess whether the conclusions of this study would apply to other situations using different amendments (e.g. from other plant, manure, and compost sources) and other agricultural soils under field conditions. Results from such studies would be useful for determining the best amendments for building soil structure in a wide range of soils and conditions.

**Table 2.1.** Chemical characteristics, including total organic C (TOC) and a proximal C fractionation, of the three organic amendment materials used in the study.

| Amendment Characteristic | Units | Amendment | | |
| --- | --- | --- | --- | --- |
| | | Compost | Manure | Vetch |
| TOC | (%) | 17.4 | 43.9 | 40.9 |
| Nonpolar C | (% of TOC) | 2.2 | 3.9 | 3.2 |
| Water Soluble C | (% of TOC) | 7.1 | 11.4 | 24.9 |
| Acid Soluble C | (% of TOC) | 17.1 | 21.3 | 29 |
| Lignin & Humic C | (% of TOC) | 73.6 | 63.4 | 42.9 |
| Total N | (%) | 1.4 | 2.6 | 4.0 |
| C:N Ratio | | 12.4 | 16.9 | 10.2 |
| Ca | (g kg$^{-1}$) | 20.0 | 30.3 | 9.9 |
| K | (g kg$^{-1}$) | 6.7 | 11.7 | 28. 9 |
| Mg | (g kg$^{-1}$) | 5.2 | 10.4 | 2.4 |
| P | (g kg$^{-1}$) | 3.1 | 9.5 | 4.2 |
| Co | (mg kg$^{-1}$) | 10.0 | 2.5 | 0.4 |
| Cr | (mg kg$^{-1}$) | 27.0 | 2.9 | 0.7 |
| Cu | (mg kg$^{-1}$) | 62.2 | 136.4 | 13.9 |
| Fe | (mg kg$^{-1}$) | 11800 | 1240 | 303.5 |
| Mn | (mg kg$^{-1}$) | 363.1 | 248.3 | 90.8 |
| Mo | (mg kg$^{-1}$) | 1.0 | 3.9 | 0.4 |
| Ni | (mg kg$^{-1}$) | 26.4 | 7.9 | 1.5 |
| Zn | (mg kg$^{-1}$) | 176.6 | 1010 | 71.2 |

**Table 2.2.** Effects of organic amendments (Amend) on chemical characteristics of the soils used in the study. Each value represents the mean of three replicates at the end of the experiment (day 82), unless otherwise indicated. For each soil, means in the same column followed by the same letter are not significantly different, as determined by the Fisher's least significant difference test using an α = 0.05. Total oganic carbon is abbreviated as TOC.

| Soil | Initial | Final | Initial | Final | Total | Mehlich III Extractable Elements | | | | | | | | |
| Amend | pH | pH | TOC | TOC | N | P | K | Ca | Mg | Mn | Na | Fe | Al | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | %— | | | | | | mg kg$^{-1}$ | | | | | |
| Maury | | | | | | | | | | | | | | |
| Control | 7.30$^{ab}$ | 6.55$^a$ | 2.18$^a$ | 2.38$^a$ | 0.19$^a$ | 141$^a$ | 355$^a$ | 2451$^b$ | 171$^a$ | 191$^b$ | 22$^a$ | 163$^d$ | 921$^c$ | 8.8$^a$ |
| Compost | 7.16$^a$ | 6.61$^a$ | 3.11$^b$ | 2.98$^b$ | 0.28$^b$ | 182$^b$ | 638$^c$ | 2989$^c$ | 277$^b$ | 179$^a$ | 92$^b$ | 159$^c$ | 851$^b$ | 11.1$^b$ |
| Manure | 7.36$^b$ | 6.96$^b$ | 3.18$^b$ | 2.48$^a$ | 0.26$^b$ | 249$^c$ | 550$^b$ | 2498$^b$ | 355$^c$ | 196$^{bc}$ | 138$^c$ | 143$^a$ | 847$^b$ | 18.7$^c$ |
| Vetch | 7.33$^b$ | 6.65$^a$ | 3.13$^b$ | 2.60$^a$ | 0.29$^b$ | 156$^a$ | 861$^d$ | 1984$^a$ | 169$^a$ | 199$^c$ | 21$^a$ | 149$^b$ | 808$^a$ | 9.6$^a$ |
| Salvisa | | | | | | | | | | | | | | |
| Control | 6.52$^b$ | 5.92$^c$ | 1.19$^a$ | 1.10$^a$ | 0.13$^a$ | 320$^a$ | 94$^a$ | 2590$^b$ | 154$^a$ | 104$^b$ | 14$^a$ | 236 | 1180$^c$ | 6.0 |
| Compost | 6.46$^b$ | 5.76$^b$ | 2.08$^b$ | 2.13$^c$ | 0.21$^b$ | 370$^c$ | 190$^b$ | 3040$^c$ | 248$^b$ | 87$^a$ | 88$^b$ | 233 | 1073$^a$ | 4.9 |
| Manure | 6.90$^c$ | 5.66$^b$ | 2.28$^b$ | 1.77$^{bc}$ | 0.21$^b$ | 432$^d$ | 159$^b$ | 2883$^c$ | 332$^c$ | 110$^b$ | 125$^c$ | 229 | 1109$^b$ | 7.2 |
| Vetch | 6.34$^a$ | 5.46$^a$ | 2.17$^b$ | 1.69$^b$ | 0.22$^b$ | 358$^b$ | 391$^c$ | 2302$^a$ | 164$^a$ | 136$^c$ | 14$^a$ | 232 | 1114$^b$ | 5.3 |
| Yeager | | | | | | | | | | | | | | |
| Control | 5.43$^a$ | 4.60 | 1.27$^a$ | 1.23$^a$ | 0.08$^a$ | 26$^a$ | 45$^a$ | 210$^a$ | 42$^a$ | 50$^b$ | 6$^a$ | 272$^{bc}$ | 398$^b$ | 6.1$^a$ |
| Compost | 5.69$^{ab}$ | 4.60 | 2.15$^b$ | 2.39$^c$ | 0.17$^b$ | 108$^c$ | 341$^c$ | 1119$^d$ | 163$^c$ | 42$^a$ | 79$^b$ | 258$^a$ | 351$^a$ | 5.1$^a$ |
| Manure | 7.00$^c$ | 4.83 | 2.26$^b$ | 1.95$^b$ | 0.17$^b$ | 180$^d$ | 245$^b$ | 716$^c$ | 243$^d$ | 47$^b$ | 134$^c$ | 280$^c$ | 327$^a$ | 12.0$^b$ |
| Vetch | 5.71$^b$ | 4.74 | 2.63$^c$ | 1.90$^b$ | 0.21$^c$ | 90$^b$ | 751$^d$ | 303$^b$ | 78$^b$ | 62$^c$ | 10$^a$ | 268$^{ab}$ | 339$^a$ | 6.1$^a$ |

**Table 2.3**. Effects of organic amendments (Amend) on concentrations of fatty acid methyl ester (FAME) biomarkers in the various soils on days 30 and 82 of the incubation. Concentrations are presented for arbuscular mycorrhizal fungi (AMF), actinomycetes (Actino), fungi, bacteria and total FAMEs. Total FAMEs includes other FAMES such as those of eukaryotic origin and those that are not designated as signature biomarkers. Also presented is the ratio of fungal to bacterial fatty acid biomarkers (F:B). Each value represents the mean of three replicates. For each soil, means in the same column followed by the same letter are not significantly different, as determined by Fisher's least significant difference test using an $\alpha = 0.05$.

| Soil | Day 30 | | | | | | Day 82 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amend | AMF | Actino | Fungi | Bacteria | Total | F:B | AMF | Actino | Fungi | Bacteria | Total | F:B |
| Maury | | | μmol kg$^{-1}$ | | | | | | μmol kg$^{-1}$ | | | |
| Control | 9.3$^a$ | 13$^a$ | 32$^a$ | 80$^a$ | 302$^a$ | 0.40$^a$ | 9.0$^a$ | 13$^a$ | 26$^a$ | 76$^a$ | 241$^a$ | 0.34$^{ab}$ |
| Compost | 11$^a$ | 24$^c$ | 39$^a$ | 134$^b$ | 549$^b$ | 0.30$^a$ | 15$^b$ | 23$^b$ | 33$^a$ | 133$^b$ | 533$^b$ | 0.25$^a$ |
| Manure | 32$^c$ | 22$^{bc}$ | 125$^b$ | 185$^c$ | 758$^c$ | 0.68$^b$ | 28$^c$ | 29$^c$ | 61$^b$ | 142$^b$ | 563$^b$ | 0.43$^b$ |
| Vetch | 19$^b$ | 20$^b$ | 102$^b$ | 164$^c$ | 717$^c$ | 0.63$^b$ | 18$^b$ | 23$^b$ | 85$^c$ | 131$^b$ | 582$^b$ | 0.65$^c$ |
| Salvisa | | | | | | | | | | | | |
| Control | 5.0$^a$ | 10$^a$ | 13$^a$ | 57$^a$ | 183$^a$ | 0.22$^a$ | 4.1$^a$ | 8.4$^a$ | 11$^a$ | 47$^a$ | 152$^a$ | 0.23$^a$ |
| Compost | 9.1$^b$ | 24$^c$ | 23$^a$ | 138$^b$ | 502$^b$ | 0.17$^a$ | 9.3$^b$ | 16$^b$ | 16$^a$ | 95$^b$ | 354$^b$ | 0.17$^a$ |
| Manure | 43$^c$ | 19$^b$ | 190$^b$ | 182$^c$ | 832$^c$ | 1.04$^b$ | 40$^c$ | 19$^c$ | 117$^b$ | 145$^d$ | 629$^c$ | 0.80$^b$ |
| Vetch | 8.4$^{ab}$ | 19$^b$ | 291$^c$ | 180$^c$ | 1081$^d$ | 1.62$^c$ | 5.3$^a$ | 17$^{bc}$ | 223$^c$ | 126$^c$ | 826$^d$ | 1.76$^c$ |
| Yeager | | | | | | | | | | | | |
| Control | 4.3$^a$ | 7.7$^a$ | 6.0$^a$ | 46$^a$ | 172$^a$ | 0.13$^a$ | 3.2$^a$ | 6.7$^a$ | 5.4$^a$ | 34$^a$ | 143$^a$ | 0.16$^a$ |
| Compost | 8.7$^c$ | 19$^c$ | 16$^a$ | 110$^b$ | 423$^b$ | 0.15$^a$ | 7.0$^b$ | 14$^b$ | 13$^a$ | 86$^c$ | 357$^b$ | 0.15$^a$ |
| Manure | 15$^d$ | 13$^b$ | 173$^c$ | 133$^d$ | 673$^c$ | 1.30$^c$ | 14$^c$ | 13$^b$ | 177$^c$ | 85$^c$ | 566$^d$ | 2.12$^c$ |
| Vetch | 5.7$^b$ | 8.8$^a$ | 81$^b$ | 116$^c$ | 636$^c$ | 0.70$^b$ | 3.5$^a$ | 6.9$^a$ | 45$^b$ | 69$^b$ | 418$^c$ | 0.64$^b$ |

**Figure 2.1**. Effects of organic amendments on large macroaggregate formation on days 0, 5, 12, 30, and 82 during an 82 day incubation of three soils where native soil structure was forced (by sieving) to microaggregate size (53 – 250 μm) on incubation day 0. Large macroaggregates are presented as a mass percentage of whole soil. Each bar represents the mean of three replications. For a given time period, values are not significantly different when they share the same letters above bars as determined by Fisher's least significant difference at an α = 0.05.

**Figure 2.2**. Effect of organic amendments on ergosterol concentration in the soil treatments after 0, 5, 12, 30, and 82 days of incubation. Each bar represents the mean of three replications. For a given time period, values are not significantly different when they share the same letters above bars as determined by Fisher's least significant difference at an $\alpha = 0.05$.

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**Figure 2.3**. (a) Non-metric multidimensional scaling (NMS) ordination of FAME relative abundance data at day 82 of the study. The axes correspond to those used to summarize microbial community composition and relationships with soil parameters in Figure 2.3b. (b) NMS joint plot showing relationships between microbial community composition, formation of large macroaggregates (LMA), and soil properties at day 82 of the study. Each geometric shape in Figure 2.3b represents a unique microbial community profile associated with one soil-amendment combination. The angles and lengths of vectors

indicate the direction and strength of relationships between ordination scores and variables, including amendment available C (Avail. C), large macroaggregate percentage (LMA), actinomycete FAMEs, mycorrhizal FAMEs, bacterial FAMEs, fungal FAMEs, microbial biomass (based on total FAMES), ergosterol, and Mehlich III Mg, K and P.

# CHAPTER 3

## CAN SOIL MANAGEMENT-INDUCED DIFFERENTIAL GENE EXPRESSION IN TOMATO (*SOLANUM LYCOPERSICUM* L.) BE LINKED TO MANAGEMENT-DRIVEN SHIFTS IN THE SOIL MICROBIAL COMMUNITY

**Introduction**

Soil management is a critical component of sustainable agroecosystems because it affects soil health and soil quality (Doran, 2002). Because the soil microbial community governs many key soil processes, including turnover of SOM, formation of humus, nutrient cycling, and facilitating good soil structure (Kennedy and Papendick, 1995), understanding management impacts on this community is important to those interested in sustainable management (Brussard et al., 2007). Soil management with organic inputs can affect this community. For example Larkin et al. (2006) found that dairy and swine manure caused increased bacterial populations, particularly Gram– bacteria, while Buyer et al. (2010) found that a vetch cover crop increased the proportions of phospholipid fatty acid (PLFA) biomarkers for actinomycetes, fungi, arbuscular mycorrhizal fungi, and bacteria along with microbial biomass. In addition, Saison et al. (2006) and Larkin et al. (2011) observed that compost affected microbial community compostion. The complexity of amendments can also affect the microbial community. Schutter and Dick (2001) used ester-linked fatty acid methyl esters (EL-FAMEs) to assess soils that were amended with cellulose and found them to have elevated levels of fungal biomarkers relative to soils amended with more labile substrates such as glucose or gelatin.

Recent research has produced evidence that soil management practices can also affect plant health via modulation of plant gene expression. Kumar et al. (2004) found

that, when compared to plants grown in black plastic mulch, tomatoes grown following a vetch cover crop showed a "distinct expression profile of select gene transcipts" that was a product of alternative management practice.  In their study, plants grown following vetch were observed to have increased expression of certain nitrogen responsive genes, defense response genes, hormone response genes and chaperone genes.  Kumar et al (2004) found plants that followed vetch to be healthier in that they were more resistant to disease and exhibited delayed leaf senescence.   In other studies, Lu et al. (2005) found that some nitrogen response genes in wheat were differentially expressed between manure treated soil and soil fertilized with inorganic ammonium nitrate fertilizer while Kavroulakis et al. (2006) found that tomatoes grown in soils amended with disease suppressive compost had greater expression of pathogenesis related *PR* genes, possibly conferring enhanced disease resistance to these plants.  Tenea et al. (2012) were able to differentiate between wheat plants grown in conventional soils and those grown in organically managed soils based on ten differentially expressed transcripts.

In a review of crop genetic responses to management practices that expands upon the work of Kumar et al. (2004), Matoo and Abdul-Baki (2006) note that in the "complex web of plant-soil interactions" it is likely that soil microbes play a role in eliciting favorable genetic responses in plants.  The idea that microbes in a diverse soil ecosystem can directly benefit plants (beyond the well characterized symbiotic relationships involving mychorrhizae or legume-associated nitrogen fixers) has been a key tenet of modern sustainability initiatives (Brussard et al., 2007).  The existence of microorganisms that promote plant growth has been well documented (Glick, 1995, Bent, 2006).  Hormonal compounds produced by both plants and soil microbes, such as

cytokinins and gibberellins, have been associated with plant growth promoting activity of

some soil microbes (Arshad and Frankenberger, 1998; Bloemberg and Lugtenberg,

2001). Ryu et al. (2003) found that cytokinin from soil bacteria stimulated plant growth

in *Arabidopsis* and Mattoo and Abdul-Baki (2006) suggest that cytokinin producing soil

microbes may play a role in the effect seen in the tomatoes that followed vetch in the

study by Kumar et al. (2004). In another study Harman et al. (2004) reviewed

mechanisms by which fungi from the genus *Trichoderma* promote plant growth and

disease resistance by producing compounds that affect plant proteomics and metabolism.

It has also been shown that plant growth promoting organisms can affect gene

expression in plants (Park and Kloepper, 2000; Bent, 2006). Research involving plant

growth promoting microbes has mainly involved attempts to develop inoculants or

amendments that promote growth or suppress disease (Glick et al., 1999; Sturz and

Christie, 2003). While some have examined the idea of managing native microbial

communities to develop disease suppressive soils (Mazzola, 2004), little has been done to

attempt to manage soil microbial communities *in situ* such that the population of

indigenous organisms directly promotes plant health via modulation of plant genetics.

Characterizations of the soil microbial community are difficult, involving indirect

measures that may not capture changes within critical microbial functional groups, thus

limiting the understanding of interactions between microbes, plants, and the surrounding

environment (Cortois and DeDeyn, 2012). As a result, little information is available that

relates soil microbial diversity or community dynamics to crop health. Matoo and Abdul-

Baki (2006) note that research is needed to gain understanding of the magnitude of, and

mechanisms involved in, soil microbial community impacts on crop genetic responses to

management practices. A major question spurred by the Kumar et al. (2004) study and the review by Mattoo and Abdul-Baki (2006) is this: Can soil management-influenced microbial community groups be related to genetic modulations in plants? This research was designed to begin to fill this information gap. The objective of this research was to evaluate effects of various soil management practices on the expression of selected genes in tomato and to examine differential gene expression relationships with management influenced soil microbial groups. Understanding linkages between modulations of plant genetics and management-induced shifts in microbial communities would be useful to researchers, and ultimately producers because information on such linkages could be used in developing integrated models for determination and evaluation of best management practices.

**Materials and Methods**

*Soil Collection and Treatment Preparation*

Soils (Maury silt loam: Fine, mixed, active, mesic Typic Paleudalfs) were collected, to a depth of 15 cm, from the University of Kentucky Horticulture Research Farm in Lexington, KY, on 24 March 2012. One soil was collected from a field that had been managed conventionally for at least 20 years while the other soil was collected from a field that had been managed organically since 2004. Both soils had similar soil textures (8-11% sand, 68-72% silt, and 20-21% clay), but were divergent in soil organic C, pH, and several macronutrient and micronutrient contents (see the "control" and "organic" treatments in Table 3.2, where the control is the conventional soil). Soils were passed

through an 8 mm sieve to remove coarse fragments and larger organic debris and stored at 4°C for two weeks before used to prepare treatments

Six soil management treatments were investigated during this research: 1) control (unadulterated conventional Maury), 2) vegetable compost amended 3) dairy manure amended, 4) vetch amended, 5) organically managed (unadulterated), and 6) inorganic N nitrogen fertilizer. The vetch was a winter cover crop planted in fall 2008 at The University of Kentucky Horticulture Research Farm in Lexington, KY and harvested in spring 2009. Dairy manure was obtained from the University of Kentucky Dairy Research Facility, Lexington, KY. The green-waste based compost was purchased from Peaceful Valley Organic Supplies (Grass Valley, CA). Vetch and manure were dried at 65°C, ground, and passed through a 2 mm sieve, and then allowed to equilibrate to air-dry moisture content. Compost was air-dried for 48 hours and then passed through a 2 mm sieve.

Organic materials were amended via thorough mixing with a garden trowel to the conventionally managed soils at a rate of $0.01$ g C $g^{-1}$ soil, which was equivalent to 89, 26, and 25 g amendment $kg^{-1}$ soil for compost, vetch, and manure, respectively. The inorganic N fertilizer treatment was included as a positive control for N effects on plants and consisted of weekly application of 30 mL of a solution of 50% v/v 0.005 M ammonium nitrate/0.005 M urea to achieve a weekly rate of 25 kg N $ha^{-1}$ which is ideal for tomato production (Hartz and Bottoms, 2009). While the organic amendments provided a means to examine immediate plant gene responses and microbial community responses to organic additions to soils, the organically managed soil was included to further examine plant gene responses in a similar soil whose chemical and microbial

properties had been altered through long term management with repeated applications of cover crops, manure inputs, and compost inputs.

Treated and untreated soils were mixed and allowed to acclimate at 23°C for 14 days to allow partial degradation of amendment material. After this acclimation period any amendment derived fatty acids that could interfere with the microbial community composition analysis using the fatty acid methyl ester approach should be degraded (Klamer and Bååth, 1998). To ensure adequate drainage in experimental pots, perlite was added to all soil preparations after acclimation, at a rate of 1 part perlite to 2 parts soil. Soil preparations were added to square pots (12 cm depth, 1100 cm$^3$ total volume) to within 1 cm of the rim, with three replications per treatment setup in a randomized complete block design. Three tomato (*Solanum lycopersicum* cv. M82) seeds were planted in each pot to a depth of 1 cm and watered with 200 mL distilled, deionized water. Each experimental block was placed under a separate 400 W high pressure sodium grow light (Eurosystems, Rohnert Park, CA). Light intensities at 30 cm below the lights ranged from 1000 to 1100 µmol m$^{-2}$ s$^{-1}$, as determined with a Licor LI-185B light intensity meter (LiCor, Lincoln, NE). Temperature at 30 cm below the lights was 29°C during the 14 hour photoperiod and 23°C when lights were off.

Plant emergence dates were recorded for each plant and extra plants were culled such that each pot contained only one plant. Plants were watered with approximately 200 ml of distilled, deionized water every 4 days during the first two weeks of growth, after which they were watered every three days. When weekly fertilizer was applied in the inorganic N fertilizer treatment, 170 mL of distilled, deionized water was added followed by the 30 mL of fertilizer solution. After plants emerged they were allowed to grow for

28 days.  At the end of this period, plant heights were measured, and plants and soils in the pots were destructively sampled for determination of chemical, microbial, and gene expression properties.

*Collection and Processing of Experimental Plant and Soil Samples*

Plant tissue designated for gene expression analysis was obtained on the 28th day after emergence from the first fully expanded compound leaf closest to the terminal leaflet at the top of the plant (generally the fourth or fifth leaf up from the base of the plant).  Leaves were immediately flash frozen in liquid nitrogen and stored at -80°C. The dry mass of above-ground plant tissue was determined after drying for 72 h at 60°C. Dried tissues were ground to a fine powder in a Cyclone Sample Mill (Udy Corporation, Fort Collins, CO) for total elemental analysis.

At the time that plants were sampled, 50 g of soil was also collected from pots using a cork borer (12 mm id) (Humboldt Manufacturing Co., Schiller Park, IL).  This soil was then passed through a 2 mm sieve and stored at -20°C until chemical and microbial analysis.

*Chemical analysis of soils, amendments, and plants*

Soil pH was determined from a 1:1 soil:water paste using a calibrated pH meter and electrode (Soil and Plant Analysis Council, 2000). Cation exchange capacity (CEC) and base saturation were determined by the ammonium saturation method.  Available nutrient (P, K, Ca, Mg, Zn, B, Cu, Mn, Na, Fe, and Ni) and Al concentrations in soils were determined via Mehlich III extraction followed by quantification by inductively

coupled plasma spectrophotometry. Total organic C (TOC) and total N (TN) were determined using a LECO dry combustion instrument. Permanganate oxidizable C (POXC) was measured using the method of Weil et al. (2003) as modified (using 2.5 g soil instead of 5 g soil) in Lucas and Weil (2012). Ammonium and nitrate were extracted from 5 g of soil in 25 mL of 2 $M$ KCl as described in Drinkwater et al. (1996). Ammonium concentration in extracts was determined colorimetrically at 630 nm using a modified Berthelot reaction (Chaney and Marbach, 1962). Nitrate was determined by first reducing it to nitrite using a cadmium reductor and subsequently analyzing colorimetrically at 540 nm using a Greiss reaction (Crutchfield and Burton, 1989). Colorimetric analyses were carried out using a μQuant microplate reader (BioTek, Winooski, VT).

Proximate organic C distribution of the organic amendments was determined using a fractionation procedure that segregates total organic carbon into lipid C, water soluble C, acid soluble C, and lignin and humic C pools (Ryan et al., 1990; D'Angelo et al., 2005). Total Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, P, Ni, and Zn of the organic amendments and tomato plant tissue were determined using the nitric acid digestion procedure described in D'Angelo et al. (2012). Digests were analyzed for metals and metalloids by the University of Kentucky, Division of Regulatory Services using a Varian Vista-PRO Inductively Coupled Argon Plasma (ICP) (Palo Alto, CA). Finely ground tomato material was assayed for total N via a Kjeldahl procedure (Nelson and Sommers, 1973) followed by colorimetric analysis at 660 nm using a Technicon System II Autoanalyzer (Tecnicon Corporation, Tarrytown, NY).

Chlorophyll concentration in the leaves was determined immediately below the leaf that was sampled for gene expression, using a Minolta SPAD-502 chlorophyll meter (Minolta corporation, Ltd., Osaka, Japan). Chlorophyll readings were taken at the center of three different leaflets and an average was calculated for each biological replicate. Conversion from SPAD units to chlorophyll was calculated using the following equation given for tomatoes in Shenker et al. (1992):

$$\text{Chlorophyll (mg g}^{-1}) = (\text{SPAD reading} - 6.6)/27.3$$

where the SPAD reading is the calculated average for each biological replicate.


*Fatty Acid Methyl Ester Extraction and Quantification*

The microbial community composition of soils in the various treatments was determined from the types and amounts of ester-linked fatty acid methyl esters (FAMEs) present in the soil. These FAMEs were extracted as described in Schutter and Dick (2000). The FAMEs were partitioned into an organic phase of 10 mL of HPLC grade hexane. Five mL of this FAME-laden hexane was transferred to a clean, glass, screw-top test tube and methyl nonadecanoate was added as an internal standard. The hexane was evaporated away under a stream of $N_2$ gas. Samples were dissolved in 0.2 mL of 1:1 hexane : methyl-tert butyl ether and analyzed using a Shimadzu GC-14A gas chromatograph (Shimadzu, Columbia, MD) equipped with a flame ionization detector and a Restek Rtx-1 column (100% dimethylpolysiloxane, 30 m by 0.25 μm, 0.32 mm ID) (Bellefonte, PA). Column temperature ramped from 80°C to 260°C, increasing at 3°C per minute. The temperature was then held at 260°C for 10 min. The FAMEs were identified by comparing retention times against those from the Supelco FAME mix C4-

C24 (Supelco, Bellefonte, PA) and to additional individual FAME standards purchased from Matreya, LLC (Pleasant Gap, PA).  The FAME nomenclature used is described in Schutter and Dick (2000).

The following microbial groups were investigated (signature FAMEs and references are given in parentheses): fungi (linolenic acid, 18:2ω6c, Frostegård and Bååth, 1996; Zelles, 1999), bacteria (sum of i15:0, a15:0, 15:0, i16:0, 16:1ω7t, i17:0, a17:0, 17:0, cyl7:0, 18:1ω7c, and cyl9:0; Frostegård and Bååth, 1996), actinomycetes (sum of 10Me16:0, 10Me17:0, and 10Me18:0; Zelles, 1999), and arbuscular mycorrhizal fungi (16:1ω5c, Olsson, 1999).  Bacteria were further subdivided according to Allison et al. (2005) into Gram negative bacteria (cy17:0, 18:1ω7c) and Gram positive bacteria (i15:0, i16:0). To determine the relative abundance of FAMEs or microbial groups, signature FAME amounts were divided by the sum of all FAMEs (total FAMEs) and multiplied by 100.

*Ergosterol Extraction and Quantification*

Ergosterol was extracted from soils using the method of Montgomery et al. (2000) with the modifications described in Zhang et al. (2008).  Ergosterol was partitioned into a pentane organic phase, filtered through a 0.45 μm, Teflon syringe filter (National Scientific, Rockwood, TN) and then evaporated to dryness under a gentle stream of $N_2$ gas.  Ergosterol was redissolved in 0.2 mL methanol and analyzed on a Shimadzu LC-10A HPLC system (Shimadzu, Kyoto, Japan).  A methanol mobile phase with a flow rate of 1.3 mL min$^{-1}$ was used to separate extracts on a Phenomenex Synergi 4u Hydro-RP 80A reverse phase C-18 column (4.6 x 150 mm) (Torrance, CA).  Ergosterol content

in extracts was quantified by comparing sample peak areas to a standard curve (ranging from 0.5 µg mL$^{-1}$ to 50 µg mL$^{-1}$) prepared from an ergosterol standard obtained from MP Biomedicals (Solon, OH).

*Gene Expression Analysis of Tomato Plants*

Total RNA was extracted from100 mg of leaf tissue using the Qiagen RNeasy$^{®}$ Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Tissue was disrupted in liquid N using a pre-cooled mortar and pestle. During the silica column based portion of the extraction process, samples were subjected to an on-column DNase treatment with DNase using the Qiagen RNase-Free DNase Set (Qiagen, Valencia, CA). Extracted RNA was visualized on a 1% agarose gel and then quantified and assessed for purity using a Varian Cary 50 Bio UV-Visible spectrophotometer (Varian, Inc., Palo Alto, CA) in conjunction with a Hellma TrayCell fibre-optic ultra-micro cell cuvette (Hellma, Müllheim, Germany). Sample purity was assessed using the ratio of absorbances at the 260 nm and 280 nm ($A_{260}/A_{280}$) wavelengths. Samples having $A_{260}/A_{280}$ greater than 1.8 were considered acceptable for use in qPCR. Total RNA was quantified based on the standard conversion factor of 1 absorbance unit at 260 nm = 40 µg RNA mL$^{-1}$ (Tsai et al., 2004).

The available literature was used to select target genes that play a role in tomato health through defense responses or by mediating nitrogen assimilation. Details for these genes, including their gene products and general role in tomato, are presented in Table 3.1. Actin (*Act*) and ubiquitin (*Ubi*) genes were used as internal standards for qPCR normalization. Sequences for all genes were obtained through GenBank (GenBank,

2012).  Primers for quantitative real-time polymerase chain reaction (qPCR) were

obtained through the literature or designed using Primer3Plus (Untergasser et al., 2012)

(Table 3.1).  To ensure that only the target sequence would be amplified, all primer sets

were verified using the Primer-BLAST tool at Genbank (GenBank, 2012).  All primers

had a Tm of 60°C ± 3°C.  The qPCR amplification efficiency of each primer set was

determined by creating a cDNA standard curve consisting of five dilutions of the

concentrated cDNA.  These dilutions were subjected to qPCR amplification (see below

for methods and parameters) and efficiency ($E$) was calculated based on the slope of the

standard curve according to the equation $E = 10^{[-1/\text{slope}]}$ (Pffaffl, 2001).  Efficiencies are

given in Table 3.1.  Melting curves (60°C – 95°C) were conducted and analyzed to

ensure the absence of nonspecific products and primer dimers.

To quantify gene expression total RNA was subjected to two-step qPCR.  First,

800 ng of total RNA was converted to cDNA using the Applied Biosystems High

Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA).  Conversions were

carried out according to the manufacturer's instructions in a total volume of 20 µL.

Reactions received 1 µL of the proprietary 20X enzyme mix which contains reverse

transcriptase.  Controls without the reverse transcriptase were included to check for

contamination with genomic DNA.  Reverse transcription was conducted in an Applied

Biosystems Veriti thermal cycler (Applied Biosystems, Foster City, CA) at 37°C for 1 h

followed by 5 min. at 95°C to stop the reaction.  Samples of cDNA were stored at -20°C

until the analysis of gene expression was conducted.

Quantitative real-time PCR reactions were conducted in 96-well plates.  Three

technical replications were used for each biological replication.  Reactions were carried

out using the Applied Biosystems Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. To each reaction well, 4 µL of 10X diluted template cDNA was added, followed by 10 µL of the Fast SYBR$^{\circledR}$ Green Master Mix. Forward and reverse primers were added at a 300 nM final concentration and the mixture was brought to a final volume of 20 µL with nuclease free water (Qiagen, Valencia, CA). Wells without cDNA template and wells that included product from the cDNA conversion reaction without reverse transcriptase were prepared as controls. Amplification was performed on an Applied Biosystems Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). Reaction parameters consisted of 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. Fluorescence of SYBR Green was measured at the end of each cycle. Amplicons were visualized on a 2% agarose gel to verify that they were the correct size as indicated by GenBank Primer-BLAST results.

To analyze qPCR data, cycle threshold values ($C_T$) technical replications for each biological replicate were averaged. Using $E,$ $C_T$ values for treatment within each biological replicate were expressed in terms of 100% efficient reactions according to the equation: $C_{Ti} = C_{To}*\log_2(E)$, where $C_{Ti}$ is the "ideal" $C_T$ expressed as a 100% efficient reaction and $C_{To}$ is the observed $C_T$ for a biological replicate (Gallup and Ackermann, 2006). In order to use multiple internal standard genes for qPCR normalization, per Vandesompele et al. (2002), the geometric mean of the $C_{Ti}$ values for the *Act* and *Ubi* genes was calculated. Relative expression ratios were then calculated for treatments by plugging the $C_{Ti}$ values for target genes and geometric mean $C_{Ti}$ values for the normalization genes into the Pffafl equation (Pffafl, 2001):

$$R = \frac{(E_{Target})^{\Delta C_{Ti(Target)}}}{(E_{Norm})^{\Delta C_{Ti(Norm)}}}$$

where, for a given target gene and soil treatment, $R$ is the relative expression ratio, $E$ is

the efficiency as described above, $\Delta C_{Ti(Target)}$ = ((Target gene $C_{Ti}$ in control samples) –

(Target gene $C_{Ti}$ in treatment samples)) and $\Delta C_{Ti(Norm)}$ = ((Geometric mean of

normalization gene $C_{Ti}$ in control samples) – (Geometric mean of normalization gene $C_{Ti}$

in treatment samples)).  Since efficiency correction was already carried out on $C_{To}$ values,

$E$ was set to 2 when the Pffafl equation was emplyed in this research.  In accordance with

Gallup and Ackermann (2006) and Gilsbach et al. (2006) relative expression ratios were

transformed using a $Log_2$ transformation to facilitate statistical analysis by giving data a

normal distribution.


*Statistics*

The experiment was conducted using a randomized complete block design with

three biological replicates for each soil treatment.  Treatment effects on microbial groups

and $Log_2$ transformed relative expression ratios were analyzed by ANOVA and post-hoc

Fisher's LSD means separation test. Simple Pearson's correlations and regression

analysis were used to examine relationships between plant parameters, soil parameters,

tomato gene expression and microbial biomarkers.  These analyses were carried out using

SYSTAT version 13 (SYSTAT Software, 2009).

The multi-response permutation procedure (MRPP) was used to determine

whether microbial community composition was significantly different in the soil

treatments. Non-metric multidimensional scaling (NMS) was used to explore the

interrelationships between the soil treatments, tomato gene expression, plant parameters,

and microbial community biomarkers, which were illustrated in joint-plots. MRPP and NMS analyses were carried out using PC-Ord version 5.1 (MJM Software, 2006).

## Results

*Effects of Soil Management on Soil Properties*

Soil management significantly affected chemical properties of the Maury soil (Table 3.2). Soil amended with compost, manure and vetch had from 41 to 69% greater total organic C at the start of the experiment (14 days after the amendments were added). The organically managed Maury soil also had 14% more total organic C than the conventionally managed control. By 28 days post plant emergence these differences in total organic C were still evident, but while the difference between the control and the organically managed soil was similar to that observed at the outset, the magnitude of the differences between the control and amended soils was slightly less, ranging from 35 to 56%. Compost, manure and vetch amended soils also had 33%, 42%, and 67% more total N than non-amended soils at the start of the experiment, while total N levels in the organically managed soil were similar to those in the conventionally managed control. With the exception of the nitrogen treated soil, total N values were similar across treatments at 28 days post emergence to what was observed at the outset of the experiment. In the nitrogen treated soil total N was 36% greater than that in the control soil at 28 days post emergence, while there was no significant difference in total N between these treatments at the outset of the experiment (Table 3.2). Vetch increased extractable $NO_3^-$–N while manure, vetch and the nitrogen treatment increased $NH_4^+$–N relative to other samples at 28 days post emergence.

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Relative to the control soil, CEC along with exchangeable $K^+$, $Ca^{2+}$, and $Mg^{2+}$ were significantly greater at 28 days post emergence in soil receiving organic amendment treatments (Table 3.2). In addition, manure treatment also significantly increased base saturation and exchangeable $Na^{2+}$. The organically managed Maury had significantly greater base saturation, and exchangeable $K^+$, $Ca^{2+}$, and $Mg^{2+}$ than the control, while the nitrogen treated soil had significantly reduced exchangeable $Ca^{2+}$ and $Mg^{2+}$. In general, soil treated with compost or manure as well as the organically managed soil had significantly greater amounts of Mehlich III extractable plant nutrients including P, K, Mg, Zn and several other elements relative to the control (Table 3.2). Vetch treatment increased Mehlich III P, K and B, while the nitrogen treatment increased Mn, Na, Fe, and Ni. Other Mehlich III extractable elements, including Cu, Mn, and Al were significantly reduced, when compared to the control, by 28 days post emergence with certain amendment treatments. Copper was also significantly lower in the organically managed Maury soil, relative to the control. In the nitrogen treated soil Ca, Mg, and Al were reduced (Table 3.2). Relative to the control soil, all amendments initially increased pH and the organically managed soil also had higher pH than the control. By 28 days post emergence pH had decreased in the vetch and inorganic nitrogen treatments to the point where pH in these treatments was significantly lower than that in the control treatment (Table 3.2).

*Effects of Soil Management on Soil Microbial Communities*

The effects of soil management were observed to have significant effects on the soil microbial community at 28 days post plant emergence (Table 3.3). Total FAMEs, an

indicator of microbial biomass, were significantly greater at 28 days post emergence in all amended soils as well as in the organically managed soil, relative to the control and nitrogen treated soil.  When compared to the control soil, total FAMEs were 124%, 329%, and 228% greater in compost, manure, and vetch treated soils, respectively.  The organically managed soil also had 63% more total FAMEs than the control soil. Significant effects of soil management were also seen within specific microbial groups (Table 3.3).  Bacterial FAMEs were 91%, 204%, 137%, and 56% greater in the compost, manure, vetch and organic treatments, respectively, relative to the control soil. Biomarker FAMEs associated with Gram positive bacteria were significantly greater in all amended soils, ranging from 117% greater in compost amended soil to 218% greater in manure treated soil.   Gram positive bacteria FAME levels were also 75 % greater in the organically managed soil (Table 3.3).  Gram negative bacterial FAMEs were 318% greater in manure amended soil and 183% greater in vetch amended soil, but not significantly greater than the control in the compost treated or organically managed soils. The fungal FAME $18:2\omega6c$ increased by 1159% with manure treatment, and 623% with vetch treatment, relative to the control (Table 3.3).   Actinomycete and arbuscular mycorrhizal fungi FAMEs were also significantly elevated in all soils treated with amendments (Table 3.3).  Manure treated soil had the highest levels of actinomycete FAMEs while vetch treated soil had the most $16:1\omega5c$, an indicator of arbuscular mycorrhizal fungi.

Soil management also significantly affected soil concentrations of ergosterol, a fungal biomarker (Table 3.3).  Soil treated with manure or vetch, along with the organically managed soil, had significantly greater ergosterol concentrations than the

control or inorganic nitrogen treated soils. Ergosterol concentration was highest in vetch treated soil, followed by manure treated soil and then the organically managed soil.

*Effects of Soil Management on Tomato Plant Characteristics*

Management treatments had significant effects on tomato plant characteristics (Table 3.4). While plants grown in the control soil and the organically managed soil were not significantly different, plants grown in soils that were amended with compost, manure, or vetch were 45%, 27%, and 46% taller, respectively, than plants in the control. Plants grown in the nitrogen treated soil were 19% taller than plants grown in the control soil. Dry biomass was highest in plants grown in vetch treated soil, followed by plants grown in manure and compost treated soil. Biomass was lowest in plants grown in the control, organic, and nitrogen treated soils. Plants grown in the organically managed soil had the lowest chlorophyll content at 28 days post plant emergence, but they were not significantly different from plants grown in the control soil. Plants grown in compost amended soil had 12% more chlorophyll than those grown in the control soil, while those grown in nitrogen treated and vetch treated soils had 16% and 41% more chlorophyll, respectively, than the control (Table 3.4). Tomato plants grown in compost and manure treated soils had lower Ca levels than the other treatments. Manure treated soil also produced tomato plants with lower Cd levels while those grown in organically managed or nitrogen treated soils had significantly more Cr. Vetch treated soil produced plants having K and Mn levels that were significantly higher than those in all other treatments except the nitrogen treated soil. Tomato plants grown in manure treated soil or the organically managed soil had significantly lower Mn levels and significantly higher Mo

levels than plants from most other treatments (Table 3.4). Plants grown in nitrogen treated soils had 79% more Ni than those from the control soil and they had significantly greater Ni content than plants from all other soil treatments. Tomato plants grown in vetch and nitrogen treated soil had 31% and 129% more Kjedahl N, respectively, than plants grown in the control soil, and Kjeldahl N levels in plants from these treatments were significantly higher than levels observed from all other treatments. The organically managed soil produced plants that had higher Kjeldahl P than the other treatments (36% greater than plants grown in the control soil) while plants from compost treated, vetch treated and nitrogen treated soils had the lowest Kjejdahl P contents.

*Effects of Soil Management on Tomato Gene Expression*

Real-time qPCR results indicate that at 28 days post emergence, soil management had significant effects on some of the target genes investigated (Figure 3.1). Compared to the other treatments, the nitrogen assimilation gene *GS1* was significantly downregulated in plants grown in nitrogen treated soil (Figure 3.1a). Significant differences due to soil management were not seen in other nitrogen assimilation genes (Figures 3.1b, 3.1c, 3.1d). Of the defense related genes investigated, all but *PAL6* showed significant effects due to soil management (Figures 3.1e-3.1i). The *ChiB* gene was downregulated, relative to the control, in plants from the manure amended soil and the organically managed soil (Figure 3.1e). In addition *ChiB* expression was not significantly upregulated when compared to the control, but gene expression in this treatment was significantly higher than that observed in plants from the manure and organic treatments. The *Osm* gene was significantly downregulated in plants from the

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organically managed soil, relative to those from the control. In plants from the nitrogen

treated soil, *Osm* was significantly upregulated relative to plants from the manure and

organic treatments (Figure 3.1f). Plants grown in the compost, nitrogen, and vetch

treated soils had significantly greater expression of the *PR1b* gene than those from other

treatments (Figure 3.1g). Plants from the compost treatment had greater *PR1b* expression

than all other plants except those from the vetch treatment. The organically managed soil

produced plants that had significantly lower expression of *GluA* than plants from the

control, compost, nitrogen and vetch treated soils (Figure 3.1h). Compost treatment

produced plants that had significantly greater expression of *GluA* than plants produced in

the manure and organic treatments.


**Discussion**

*Effects of Soil Management on Soil Properties and Plant Characteristics*

It was expected that soil management treatments would have varied effects on soil

properties. Organically managed soils have previously been shown to have greater SOC

(Pulleman et al, 2003; Marriot and Wander, 2006) than intensively tilled conventionally

managed soils. This trend was also observed in the results of this study (Table 3.2). The

organic soil has historically received greater amounts of organic inputs, including cover

crops, manure, and compost, which increase SOC and contribute nutrient inputs as

observed in the P, K, Ca, Mg, Zn, Mn, and Fe contents of the organically managed

Maury. The pH, exchangeable Ca, Mg, and K were also greater in the organically

managed soil, relative to the control. This is not surprising given that inputs used in

organic systems such as compost or manure can increase pH and contribute exchangeable

cations, depending on their source (Butler and Muir, 2006). The inorganic N treated soil was not expected to differ greatly from the control aside from N content and these expectations were generally confirmed (Table 3.2). The slight reduction in pH in the N fertilized soil seen after 28 days is not surprising given that ammonium nitrate and urea are both well known for acidifying soils through the conversion of ammonium to nitrate. As expected, total N was greater in the N fertilized soil relative to the control, with most of the increase being in the form of $NH_4^+$.

The compost, manure and vetch amendments had varying elemental compositions and varying amounts of total C, labile C constituents, recalcitrant C constituents, and total N (Table 3.5). Amendments also differed in elemental composition. Differences in amendment composition led to significant differences in soil chemical properties within amendment treated soils. All organic amendments increased Mehlich III P and K in soil. Compost increased Mehlich III Ca and Fe and manure increased Mehlich III Ni. Both manure and compost increased Mehlich III Mg, Zn, and Na. Although there are some discrepancies (for example, Ca is highest in the compost treated soil but the manure amendment actually had more Ca than the compost) the differences in soil chemical properties seen in Table 3.2 generally reflected differences in elemental composition of the amendments seen in Table 3.5. Discrepancies might be attributable to different extraction processes operating on differing matrices. Similar to the inorganic N treatment, pH declined in the vetch treated soil. As with the inorganic N treatment, this decline is most likely due to acidification as ammonium is converted to nitrate. All organic amendments increased CEC, likely due to the additional carboxyl groups being provided with the organic materials. The compost produced the greatest increase in CEC

which is not surprising given that humified materials have many of these carboxyl groups in their chemical structure. The pH effect on CEC appears to be minimal within the pH range seen in the soil treatments because the vetch and manure treated soils, while having significantly different pH after 28 days of plant growth, do not have significantly different CEC. A similar trend is seen between the control soil and the organically managed soil. At the outset of the experiment, all organic amendments had increased total organic C (TOC) in the conventional Maury soil (Table 3.2). By 28 days post plant emergence, the TOC levels in the compost, manure, and vetch treatments had decreased by 5%, 9%, and 7%, respectively, relative to the levels seen at the outset of the experiment. This likely stems from the microbial oxidation of the more labile carbon components that comprise these amendments, represented by the lipid C, water soluble C, and to a lesser degree the acid soluble C fractions in Table 3.5. The compost treatment showed the least decline in TOC because it had the greatest amount of recalcitrant lignin and humic C. These results indicate that a significant portion of the C in the manure and vetch treatments was readily available to microbes.

It was anticipated that treatment driven differences in soil properties would lead to differences in tomato plant health characteristics and the results confirm these expectations (Table 3.4). Based on soil test results and the guidelines given in Coolong et al. (2012) nutrient levels were sufficient for tomato production in all treatments (Table 3.2). In particular, soil P and K levels rated "high" to "very high" according to Coolong et al. (2012). In addition, Mg levels in all soil treatments were well above the 99 mg kg$^{-1}$ (224 kg ha$^{-1}$) indicated as minimally sufficient. Given that all other plant available nutrients were adequately supplied, most of the differences seen in plants were probably

driven by treatment influenced soil nitrogen levels (Table 3.2). Plants grown in

amendment treated soils were generally taller and produced more biomass than plants

from the control, nitrogen treated, or organically managed soils. Plants from vetch

treated soil had significantly more chlorophyll (Table 3.4) than plants from all other

treatments. This makes sense, given that vetch treated soil had the highest levels of total

N, and $NO_3^-$–N and also had significantly higher $NH_4^+$–N than all treatments except the

manure treatment (Table 3.2). Plants from the vetch treated soil had higher Kjeldahl N

than all but the plants from the nitrogen treated soils. Interestingly, plants from the

organically managed soil had the lowest chlorophyll levels, being significantly lower than

levels seen in plants from the vetch, nitrogen, and compost treatments (Table 3.4). This

is a curious result because others have found that compared to conventional management,

organic management practices increase particulate organic matter N (Marriot and

Wander, 2006), which is labile and is related to mineralizable N (Magid and Kjærgaard,

2001). With this in mind it was expected that the organically managed Maury soil would

at least have more plant available N than the control soil. The organically managed

Maury may be a case of a finer textured soil, having a higher SOM content than its

conventionally managed counterpart, but also having lower amounts of available N

because SOM derived N can become stabilized in organomineral complexes. Magdoff

(1991) observed such phenomena while conducting field assessments of nitrogen

dynamics to predict N availability to corn (*Zea mays* L.). Plant nutrient differences

generally reflected treatment influenced soil nutrient levels.

Plant health characteristics, not surprisingly, were related to soil nutrient status.

Plant height (r = 0.64, P = 0.004), biomass (r = 0.82, P < 0.001), and chlorophyll content

(r = 0.67, P = 0.002) were positively correlated with total N (Appendix B, Table B1).

Chlorophyll content was also correlated (r = 0.56, P = 0.017) with Mehlich III soil Ca.

Plant height (r = -0.63, P = 0.005) and biomass (r = -0.69, P = 0.002) were negatively

related to Mehlich III Al. While this result would not be unexpected in soils having a low

pH and potential aluminum toxicity problems, it is unlikely that aluminum had

detrimental effects on plants in this study because Al availability should be low within

the soil pH range observed in this study (Brady and Weil, 2002). A likely explanation is

that in soils treated with organic amendments, the decmposing organic materials formed

complexes with aluminum (Mortensen, 1963), further reducing availability (Table 3.2),

while also producing larger and taller plants via nutrient supplementation. In the control

and organically managed soils Al remained unchanged and plants received no nutrients to

spur more vigorous growth. Correlations with $NO_3^-$–N and $NH_4^+$–N could not be

interpreted because the data clustered into two distinct groups (the clustering was

primarily due to the large increases in N parameters associated with the vetch and manure

treatments). Plant biomass was positively correlated to both TOC (r = 0.73, P = 0.001)

and POXC (r = 0.68, P = 0.002). Plant height was related to these soil C parameters as

well (TOC: r = 0.48, P = 0.042; POXC: r = 0.52, P = 0.026). The relationships between

plant health characteristics and soil C measurements likely stems from the coinciding

nutrient inputs associated with the amendments.


*Effects of Soil Management on Soil Microbial Communities*

Given the results of Chapter 2, it was expected that soil management treatments

would have significant effects on the soil microbial biomass and community structure.

Total FAME concentrations, an indicator of microbial biomass (Zelles, 1999), were greater in all amendment treatments and in the organically managed soil (Table 3.3).  The resuklts of Chapter 2 of this dissertation showed a similar trend with amendment treatment across three soils and other researchers have also observed increases in microbial biomass with organic amendments and in organically managed systems (Frostegård et al., 1997; Peacock et al., 2001; Esperschütz et al. 2007; Buyer et al., 2010).

Microbial group signature FAMEs generally increased in amended soils (Table 3.3).  Concentrations of indicator FAMEs for actinomycetes, arbuscular mycorrhizal fungi, bacteria and Gram positive bacteria were elevated relative to the control in all amendment treatments.  Relative to the control soil, manure treated soil and vetch treated soil also had elevated fungal and Gram negative bacterial FAMEs.  The results of this research agree with Buyer et al. (2010), who found that signature FAMEs for actinomycetes, fungi, arbuscular mycorrhizal fungi, and bacterial groups increased following a vetch cover crop.  These results also agree with other studies that have shown significant increases in bacterial, Gram negative, Gram positive, and fungal FAMEs in manure treated soils (Peacock et al., 2001; Larkin et al., 2006; Zhong et al., 2010).  The findings of Moeskops et al. (2012) contrast with this study in that they saw no significant increases in fungal FAMEs with manure treatment.  In compost amended soils, Larkin et al. (2011) saw increases in mycorrhizal FAMEs, while Saison et al. (2006) saw increases in bacterial and fungal FAMEs.   The findings of Larkin et al. (2011) are similar to the findings of this study and the results of Chapter 2, while those of Saison et al. (2006) contrast with the findings of this study.

The results indicated that treatment with inorganic N did not lead to significant differences in any microbial group relative to the control soil (Table 3.3). These results agree with both Moeskops et al. (2012) and Zhong et al. (2010) who did not see differences in signature FAMEs between fertilized and non-fertilized soils. Signature FAME concentrations, in general, tended to be greater in the organically managed Maury soil relative to the control, but significant differences were only evident in bacterial FAMEs and in FAMEs associated with Gram positive bacteria. Esperschutz et al. (2007) found, in fields that had been managed conventionally or organically since 1978, that bacterial and fungal FAME concentrations were increased in the organically managed soil. Another study (Moeskops et al., 2010) found fatty acids representing all microbial groups had significantly higher concentrations in organically managed soil. Given the general trends seen in signature FAMEs from the organically managed Maury in this work, it is likely that differences between these results and those of Esperschutz et al. (2007) and Moeskops et al. (2010) may be related to the duration of organic management at the various locations. The soil in this study has been managed organically for 8 years while the soil in Esperschutz et al. (2007) had over 25 years of organic management history. The fields used by Moeskops et al. (2010) ranged from as low as 3 years to as long as 23 years of organic management.

Changes in FAME profiles between treatments were likely driven by differences in the C composition of the various amendments (Table 3.5), or in the case of the organically managed soil, the C content of the soil in general (Table 3.2). As observed and discussed in Chapter 2, vetch and manure provided more labile and semi-labile C to soil microorganisms as indicated by the water soluble and acid soluble C pools in the

proximal fractionation (Table 3.5).  The organically managed soil had greater TOC and POXC than the control (Table 3.2), which likely stimulated the greater microbial biomass (Total FAMEs; Table 3.3) observed.

The soil ergosterol levels at 28 days post plant emergence supported the fungal FAME biomarker results.  Similar to the signature FAME, ergosterol levels were highest in the vetch-treated soil and the manure-treated soil, and lowest in the control soil, compost-treated soil, and nitrogen-treated soil (Table 3.3).   Similar to the results of this study, Quintern et al. (2006) did not find any significant increase in ergosterol in soil amended with compost and Dinesh et al. (2009) saw increased ergosterol in soils under leguminous cover crops.  The increases in $18:2\omega6c$ concentrations were very large in the manure and vetch treated soils while increases were not seen in other management treatments, relative to the control (see Fungi in Table 3.3).  This caused data to lump into two clusters when ergosterol vs. $18:2\ \omega6c$ was analyzed using Pearson's correlations. This data clustering rendered the correlation analysis inconclusive.

While the fungal signature FAME in this study was highest in the manure treated soil, ergosterol was highest in the vetch treated soil (Table 3.3).  Hogberg (2006) suggests that discrepancies between the $18:2\omega6c$ and ergosterol biomarkers may be related to differences in the decay rates of these compounds.  Such discrepancies could become magnified when acting in conjunction with different decay rates of amendments.  These results would be worrisome if the vetch treatment caused a greater than expected spike in $18:2\omega6c$, which could indicate interference from plant FAMEs.  The fact that the fungal FAME biomarker and ergosterol results do not drastically differ supports the assumption

that the FAME biomarker is indeed representative of fungi without being confounded with plant derived fatty acids.

This experiment was designed such that shifts in microbial FAME profiles would be driven primarily by carbon differences between soil management treatments. Because it is often a limiting nutrient in soils, it was also anticipated that nitrogen could play a role in microbial community dynamics. In its role as a "master variable" controlling nutrient availability it was expected that pH may also affect microbial community composition. Pearson's correlations between the concentrations of individual biomarker FAMEs and TOC, POXC, extractable $NH_4^+$ –N, extractable $NO_3^-$–N, total N and pH were examined across all management treatments for relationships between microbial parameters and soil parameters. Relationships between 18:2ω6c and 18:3ω3 and soil parameters could not be determined because concentrations of these FAMEs in manure and vetch treated soils were much greater than those from other treatments causing the data points to cluster into two distinct groups. Compared to other treatments, extractable $NH_4^+$–N was also much higher in the manure and vetch treatments and $NO_3^-$–N was very high in the vetch treatment (Table 3.2), causing data clustering problems and preventing conclusive correlation analysis with these parameters. The strongest relationships between FAME concentrations and soil parameters were always seen with TOC (ranging from r = 0.72, P = 0.001 with cy17:0 to r = 0.97, P < 0.001 with 15:0), and POXC (ranging from r = 0.72, P = 0.001 with cy19:0 to r = 0.92, P < 0.001 with i15:0). Aside from FAMEs where relationships could not be determined, only the 10Me17:0 and 20:4ω6 FAMEs were not correlated with soil C parameters. Relationships between FAMEs and total N (ranging from r = 0.48, P = 0.042 with i17:0 to r = 0.75, P < 0.001 with 18:1ω5c) or pH (ranging

from r = 0.49, P = 0.039 with i17:1ω7c to r = 0.58, P = 0.012 with i17:1ω7c) were observed but, within the same FAME, none of these relationships were as strong as corresponding relationships with soil C parameters. Not surprisingly total FAMEs were also strongly related to both soil C parameters (Figures 3.2a, 3.2b) indicating clearly that microbial biomass increased with increasing amounts of soil organic carbon. Total FAMEs were related less strongly with total N (r = 0.60, P = 0.009). Ergosterol was also correlated strongly with both TOC (r = 0.78, P < 0.001) and POXC (r = 0.86, P < 0.001) while showing a weaker relationship with total N (r = 0.66, P = 0.003). Neither total FAMEs nor ergosterol were related to pH.

In amendment treated soils, TOC is a function of the amendment C introduced to the soil. The FAME profiles associated with amendment treatments are products of the amounts of labile and semi-labile C compounds that comprise each amendment (Table 3.5). Because of the recalcitrant nature of humus (Stevenson, 1994) and lignin (Swift et al., 1979) the majority of the amendment C utilized by microbes would be expected to be derived from the three labile or semi-labile C pools (nonpolar, water soluble, and acid soluble) comprising the amendment C fractionation. These pools make up 26%, 37% and 57% of the C in compost, manure and vetch, respectively. Permanganate oxidizable C has been used to estimate a labile pool of soil carbon (Weil et al., 2003) and the amounts of labile amendment C are reflected in the permanganate oxidizable C levels seen in amendment treated soils in Table 3.2. Visual observation of the dairy manure indicated that it contained large amounts of undigested plant materials. Thus the vetch treatment and manure treatment likely stimulated fungal proliferation because these amendments contained large amounts of intact plant cell wall materials. The cellulose from these

materials is part of the acid soluble fraction in Table 3.5 and is a substrate that fungi can metabolize via production of unique extracellular cellulases, hemicellulases, and Mn-peroxidases (Muller et al. 1988; Carlile et al., 2001).

While observing concentrations of FAMEs is useful for seeing how specific groups are responding in a treatment, the relative abundance of FAMEs allows observation of community shifts. When FAME relative abundances are examined, it is clear that microbial community profiles associated with various treatments are different. Analysis of the microbial group abundances using MRPP revealed that treatments had significantly different microbial community compositions (P<0.0001; within group agreement = 0.55). The relative abundance of bacteria was significantly lower, and the relative abundance of fungi was significantly higher in the vetch- and manure-amended soils compared to control, nitrogen treated, organically managed, or compost amended soils (Table 3.3). This phenomenon is clearly reflected in the calculated fungal to bacterial ratios in Table 3.3. Bacterial relative abundance was highest in the control soil and the organically managed soil. Gram positive bacterial relative abundance was lowest in the vetch and manure treated soils while Gram negative relative abundance was greatest in the organically managed soil followed by the control and manure treated soils. Gram negative relative abundance was lowest in the compost treated soil (Table 3.3).

Differences in soil microbial community structure due to soil management can be observed in the NMS ordination of FAME relative abundance data (Figures 3.a and 3.b). In this analysis, 91% of the variation in microbial communities is explained by axis 1 while 3% is explained by axis 2. Figure 3.3a shows the ordination of individual FAMEs relative to each axis. FAMEs that were strongly correlated with axis 1 included 18:2ω6c,

18:1ω9c, 20:5ω3 (associated with fungi), and 18:1ω5c (bacteria) while the i15:0, 15:0, i16:0, i17:0, a17:0, and cy19:0 FAMEs (all bacterial) were negatively correlated to this axis.  Also negatively correlated with axis 1 were the 14:0, 16:0, 20:0, 22:0, and 24:0 FAMEs (universal FAMEs or FAMEs not designated as a signature microbial FAME) as well as the 16:1ω5c arbuscular mycorrhizal FAME and the 10Me16:0 actinomycete FAME.  FAMEs that were strongly correlated to axis 2 included 14:0, i15:0, a15:0, 15:0, 16:1ω7c, i17:1ω7c, 10Me16:0 and i17:0, while 18:2ω6c and 18:1ω5c had strong negative correlations with this axis.

The ordination of the FAMEs in Figure 3.3a determines the ordination of the individual, treatment associated microbial communities in Figure 3.3b.  The NMS joint plot (Figure 3.3b) clearly showed that microbial communities were different with differing soil management and suggests these differences are related to carbon and nitrogen.   Microbial communities associated with vetch and manure treatments ordinated close to one another, along axis 1 in the same general direction as the fungal 18:2ω6c FAME (Figures 3.a and 3.b), indicating that these treatments promoted soil microbial communities that were enriched in fungi.  Microbial communities associated with the other treatments ordinate at the opposite end of axis 1, indicating that they are quite different from the vetch- and manure-associated microbial communities.  Ordinating in the same general direction as the vetch- and manure-associated microbial communities were vectors representing total soil N, TOC, permanganate oxidizable C, ergosterol, microbial biomass (total FAMEs), fungal FAMEs, and $NH_4^+$–N.  These vectors indicate relationships between the microbial communities and the soil parameters.  Vectors representing bacterial relative abundance and Gram negative bacterial abundance

ordinate in the opposite direction from the manure and vetch influenced microbial communities, clearly showing that when fungi proliferate in the vetch or manure treatments, bacteria are proportionally reduced.   The NMS ordination suggests that the carbon and nitrogen contributions from manure and vetch spurred microbial proliferation. Fungi, in particular, are favored by addition of these amendments.  The ergosterol vector indicates a strong relationship with the vetch influenced microbial communities and its general direction is more similar to the fungi vector than any other microbial group vector, supporting the observations seen with the fungal FAME biomarker.  These results are in general agreement with results from Chapter 2.

*Effects of Soil Management on Expression of Selected Tomato Genes*

A primary focus of this research was to examine the impacts of soil management on the expression of health related genes in tomato plants.  Nine plant health related genes were investigated in this study.  Four of these genes play a role in nitrogen assimilation (*NR, NiR, GltS, GS1*) and five are defense related genes (*ChiB, GluA, Osm, PAL6, PR1b*).

Nitrate is the most common plant available form of nitrogen in soils.  Tomatoes, like all higher plants, employ a nitrate reduction pathway to assimilate nitrate from soil. The first step in this process is the conversion of nitrate to nitrite via nitrate reductase, followed by the conversion of nitrite to ammonium via nitrite reductase (Oaks, 1994). Glutamine synthetase then catalyzes a reaction between glutamate and ammonium to form the amino acid glutamine.  Finally glutamate synthase catalyzes the reaction between glutamine and 2-oxoglutarate to form 2 glutamate molecules which can then be

used in further ammonium assimilations or in other metabolic paths (Forde and Lea, 2007). Four genes coding for the enzymes in the nitrate reduction pathway were examined in this study (Table 3.4). Given that several of the soil management treatments investigated in this study affected total N, $NO_3^-$–N and/or $NH_4^+$–N in the soil (Table 3.2) it was expected that nitrogen assimilation genes would show show responses to these treatments.

After 28 days of growth in treated soils only the *GS1* gene coding for glutamine synthetase showed significant effects due to soil management (Figure 3.1a). Relative to all other treatments, the *GS1* gene exhibited reduced expression in plants from nitrogen fertilizer treated soil. The results in *GS1* are somewhat difficult to interpret and the available literature provides more questions than clarity. Among the confounding issues are the facts that there are different isoforms of *GS1* expressed within plants (Lam et al., 1996) and that *GS1* genes may behave somewhat differently across plant species (Miao et al., 1991; Ishiyama et al., 2004). Several studies have shown *GS1* to be differentially expressed at different levels of nitrate or ammonium (Ishiyama et al., 2004; Ruzicka et al. 2010; Zebarth et al. 2011; Zebarth et al. 2012). However among the multiple *GS1* genes observed in non-leguminous plants some *GS1* genes may be upreguated when plants are fertilized with ammonium or nitrate, while others are not affected (Sukanya et al.; 1994).

One reason that *GS1* may have produced lower amounts of transcript in plants from inorganic N fertilized soil is that the plants may simply have had adequate N. Plants from this treatment had the greatest Kjeldahl N among all treatments in this study (Table 3.4). Finnemann and Schjoerring (1999) found that *GS1* expression and glutamine synthetase activity were reduced in oilseed rape (*Brassica napus* L.) in their "N-replete"

plants. Zhao and Shi (2006) saw a similar pattern in some (but not all) *GS1* encoding genes when rice (*Oryza sativa* L.) was supplied with inorganic N. In other treatments in this study, *GS1* expression was not significantly different from the control, suggesting that *GS1* is down regulated in the inorganic N treatment. The literature on *GS1* expression is not consistent as others have seen increased expression of *GS1*, even at high rates of N fertilization (Ruzica et al., 2010; Zebarth et al. 2011).

Studies examining the expression of plant nitrogen assimilation genes have mainly focused on various inorganic N sources (Ishiyama et al., 2004; Ruzicka et al. 2010; Zebarth et al. 2011; Zebarth et al. 2012; others), while experiments studying gene expression response to specific soil management regimes or organic amendments are sparse in the literature. These results agree somewhat with two studies that did address responses to organic soil inputs. Kumar et al. (2004) observed that tomatoes that followed vetch had greater *GS1* expression than those grown with black plastic mulch and inorganic N fertilizer. Using microarrays, Lu et al. (2005) found that, *GS1* was expressed more in wheat (*Triticum aestivum* L.*)* from fields that received farmyard manure when compared to plants from fields that received inorganic N fertilizer. Kumar et al (2004) conclude that their results in tomato *GS1* expression are part of a greater "fingerprint of hairy vetch based alternative agriculture" and they assert that this upregulation is a sign of improved nitrogen use efficiency and mobilization within the plants. Similarly, Lu et al. (2005) state that certain gene expression patterns may be characteristic of organic fertilizer use and, with additional research, may be exploited to maximize N use efficiency in alternative systems. In conjunction with their overall results, the *GS1* results in Kumar et al. (2004) and those of Lu et al. (2005) seem like

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promising indications of soil management inducing favorable genetic responses within the crop.  However, it is also possible that, given that their black plastic mulch treatment received twice as much inorganic N fertilizer (urea at 200 kg ha$^{-1}$ in the black plastic treatment) as the vetch treatment, the *GS1* results of Kumar et al. (2004) were simply due to *GS1* repression in sufficiently fertilized plants.  It is less likely that the results of Lu et al. (2005) could be due to reduced expression as a result of fertilizer sufficiency because, in their study, in contrast to the results of this research, *GS1* was expressed more in the farmyard manure treatment relative to all other treatments, including a zero N treatment.

It is curious that no other nitrogen assimilation genes showed significant effects due to soil management.  Others have observed gene expression differences in N assimilation genes with organic or inorganic N treatments.  In *Arabidopsis thaliana* L., Wang et al. (2003) found orthologous genes to *NR, NiR,* and *GltS* to be induced  by nitrate fertilization.  Another study (Wang et al., 2001) used microarray analysis to observe *NR* and *NiR* induction by nitrate in tomato.  Lu et al. (2005) observed increased *NR* expression in wheat grown in soil that received farmyard manure relative to wheat from soil that received inorganic N treatments.  Kumar et al. (2004) observed increased *NiR* expression in tomatoes from vetch treated soil, relative to those grown in soil treated with black plastic mulch and inorganic N fertilization.  Fatima et al. (2012) observed increased *NR* expression in tomatoes following vetch relative to those grown in soil that had not received vetch.  In other studies researchers saw increases in response to varied inorganic N fertilizer levels in one or more of the genes investigated in this study. Zebarth et al. (2012) saw greater *NR* expression in potato (*Solanum tuberosum* L.) with greater $NO_3^-$ fertilization, but *NR* expression was reduced with increased $NH_4^+$.  In an

earlier study Zebarth et al. (2011) also observed that *NR* expression was reduced at the highest levels ($\geq$ 200 kg N ha$^{-1}$) of ammonium nitrate.  In addition, Zebarth et al. (2011) and Zebarth et al. (2012) observed elevated *GltS* expression with high N fertilization. Zebarth et al. (2012) and Ruzicka et al. (2010) also saw greater *NiR* expression in tomato at greater N fertilization levels.  In contrast, in the present study manure, vetch, and the inorganic N treatment increased soil N parameters, but this did not translate into observable responses in the *NR, NiR,* or *GltS*.

The differences between studies could be due to differences in plant species used and experimental conditions.  Zebarth et al. (2011) and Zebarth et al. (2012) examined gene expression in potato in soil and in hydroponic nutrient solution, respectively. Kumar et al. (2004) and Fatima et al. (2012) studied field grown tomatoes, while Ruzicka et al. (2010), similar to this study, grew tomatoes in a field soil that had been moved to a controlled indoor environment.  Lu et al. (2005) examined wheat in the famous research fields at Rothamstead, UK.  All of the studies previously mentioned examined gene expression in leaf tissues except those of Ruzicka et al. (2010) and Wang et al. (2003) which examined roots.  Examination of these genes may benefit from being carried out in roots because this is where nitrogen assimilation primarily occurs and where these genes show stronger responses to nitrogen fertilization (Lam et al. 1996; Wang et al., 2003). Attempts were made to extract roots from soil in this experiment but the fine texture of the soil prevented extraction without significant damage to the root system.  This damage could potentially confound results by inducing wounding responses in the plant (Zhou and Thornburg, 1999), thus gene expression was measured in leaves.

Based on the results of previous studies addressing defense gene responses to a single amendment (Kumar et al., 2004; Kavroulakis et al., 2006), defense gene modulation was expected in response to the various amendment treatments used in this study. Of the defense genes studied in this experiment, four (*ChiB*, *Osm*, *GluA* and *PR1b*) showed significant differences in gene expression due to soil management after 28 days of plant growth (Figures 1.e – 1.h). The *ChiB* gene encodes basic chitinase and *GluA* encodes a β-1,3-glucanase, both of which act as defense proteins against fungal pathogens by attacking the complex cross-linked carbohydrate structure of fungal cell walls (van Loon et al., 2006). An osmotin protein is encoded by *Osm* and acts against pathogens by creating transmembrane pores in their plasma membranes and also via β-1,3-glucanase-like action (Ferreia et al., 2007). The *PR1b* gene encodes the PR1b pathogenesis-related protein. The mechanism by which the PR1b protein acts in plant defense is unclear (Rivière et al., 2008), however it is thought to play a role in signaling or regulation in salicylic acid mediated systemic acquired resistance (SAR) (Agrawal et al., 2001; Diaz et al., 2002) and the *PR1b* gene is one of the most commonly induced genes in response to pathogens or stress (Hong & Hwang, 2002).

While the statistical significance of the results may vary slightly from gene to gene, the *ChiB*, *Osm*, and *GluA* genes show very similar patterns of expression across soil management treatments. These genes all have reduced transcript levels in plants from the organically managed soil, relative to plants from the control and nitrogen treatments (Figures 1.e, 1.f, 1.h). Plants from the manure treated soil also have significantly less *ChiB* transcript than plants from the control and nitrogen treatments, significantly less *Osm* transcript than plants from the nitrogen treatment, and significantly less *GluA*

transcript than plants from the compost treatment.  None of these genes were differentially expressed in plants from the compost, nitrogen or vetch treatments, relative to the control.  According to the ATTED-II database, *Arabidopsis thaliana* L orthologs to *ChiB*, *Osm*, and *GluA* are coexpressed with each other (Obayashi et al., 2007).  The similar gene expression patterns across treatments in the results suggest that these genes may be similarly coexpressed in tomato.  Interestingly, the *PR1b* gene was expressed at significantly greater levels in plants from the compost, nitrogen, and vetch treatments while control, manure, and organic treatments were not significantly different.

Many studies have linked defense gene expression to specific pathogens (Gibly et al., 2004; Balaji et al 2008; others), specific elicitors (Song et al., 2011), plant growth promoting rhizobacteria (Park and Kloepper, 2000; Bent, 2006) and even extracts from other plants (Medeiros et al, 2009), but there have been relatively few examples in the literature of studies that have examined the effects of soil management on defense genes in crops.  Kumar et al. (2004) found *ChiB* and *Osm* to have a "higher steady state of expression" in tomato plants that followed vetch relative to tomatoes grown with black plastic mulch and inorganic N fertilization.  The *PR1a* gene, which has significant homology and a similar function to *PR1b,* was also expressed at higher levels according to Kumar et al. (2004) in plants grown after vetch.  Fatima et al. (2012) compared tomatoes grown following vetch or in bare soil over a range of inorganic N fertilization levels, and saw increased *Osm* expression in tomato plants grown after vetch at all N levels, over plants grown in bare soil.  The results of this study contrast somewhat with both the Kumar et al. (2004) and the Fatima et al. (2012) studies in that vetch did not significantly increase *Osm* or *ChiB* expression in tomato plants relative to the control or

inorganic nitrogen treatments.  The results in *PR1b* expression agree with the

observations of Kumar et al. (2004) with respect to the highly similar *PR1a* gene.

Given the results of other studies, the expression pattern seen across treatments of

*Osm*, *Chib*, and *GluA*, is challenging to interpret.  One possible explanation is that these

genes may have already been at a high state of expression as a result of growing in a field

soil.  In a review article on the subject, Walters (2009) discusses the current evidence that

plants in the field are in a state of semi-constant induction through their interactions with

the soil and their general environment.  Herman et al. (2007) observed this phenomenon

in field grown tomatoes that were treated with the SAR inducer acibenzolar-S-methyl.  In

their study the inducer caused a minor induction of SAR with an initial application of the

compound.  A second application of inducer caused a much greater induction of SAR.

Herman et al. (2007) suggested that the first induction was smaller than the second

because the plants were already environmentally induced.  They also found that the

amount of induction depends on cultivar.  The M82 tomato strain, which is generally a

research/greenhouse variety, was used in this research.  While these plants were not field

grown, they were grown in field soil.  It is possible that when exposed to a field soil, the

M82 laboratory tomatoes were immediately induced by some factor, possibly a pathogen,

present in the soil.  Elevated expression of *Osm, ChiB*, and *GluA* genes has been observed

as part of the SAR process (Anfoka and Buchenauer, 1997; Lee and Hwang, 2005).  This

may explain why expression of these genes was fairly level across the compost, nitrogen,

and vetch treatments.  In other words, expression of these genes could have been at

maximum capacity through environmental induction.  Meanwhile, manure has been

shown to have disease suppressive properties (Darby et al., 2006) which may have

reduced or inhibited SAR inducing organisms in the manure treated and organically managed soils (which received manure inputs as part of their fertility regime). Reduced SAR induction may explain the reduced *ChiB, Osm*, and *GluA* transcript levels in plants from these treatments. Given that *PR1b* plays a role in SAR regulation, the increased levels of *PR1b* transcript seen in plants from the compost, vetch, and nitrogen treatments may be indicative of an environmentally induced SAR response in these treatments, while lower PR1b expression in the manure treated and organically managed soils may be indicative of reduced need for SAR response in these treatments because of suppressive activity of manure.

*Relationships Between Tomato Gene Expression and Plant, Soil, and Microbial Characteristics*

An objective of this research was to examine relationships between soil management-influenced microbial communities and gene expression in tomato plants. In order to examine these relationships, any relationships between gene expression and soil chemical parameters must also be considered (Appendix B, Table B1). In a relationship driven by the P contents of the manure treated and the organically managed soil, *ChiB* (r = -0.48, P = 0.042), *Osm* (r = -0.50, P = 0.037), and *GluA* (r = -0.63, P = 0.005) transcript levels were negatively related to Mehlich III soil P. Expression of *GluA* (r = -0.57, P = 0.014) and *Osm* (r = -0.49, P = 0.040) was negatively related to Mehlich III soil Ca and expression levels of *GluA* (r = -0.47, P = 0.05) and *ChiB* (r = -0.50, P = 0.035) were negatively related to Mehlich III soil Mg. Espression of *GS1* (r = -0.56, P = 0.017) was negatively related to Mehlich III soil Mn. Some relationships (all correlations involving

Mehlich III soil Fe or pH; *GS1* vs. soil C parameters; *ChiB* vs. soil C parameters) could not be interpreted because the data were distributed in two distinct clusters or because they were strongly influenced by one or two cases that had high leverage on the correlation coefficient. Expression of the nitrogen assimilation gene *GS1* was not significantly related to any soil N measurements. Expression of *PR1b* (r = 0.65, P = 0.003) had a strong positive relationship with total N, but not to $NO_3^-$–N or $NH_4^+$–N.

Since the nutrient status of soils was affected by soil management treatments (Table 3.2), in turn affecting nutrient uptake and plant health characteristics, (Table 3.4) it was expected that tomato gene expression may be tied to plant nutrient status. The expression *PR1b* was negatively related (r = -0.57, P = 0.015) to plant Mg content (Appendix B, Table B2). All four of the defense genes that showed significant differences due to soil management had a negative correlation with plant P content (*ChiB*: r = -0.62, P = 0.006; *GluA*: r = -0.50, P = 0.035; *Osm*: r = -0.715, P = 0.001; *PR1b*: r = -0.76, P < 0.001). The manure-treated and organically managed soils had greater Mg and P levels and plants from these treatments had greater Mg and P contents, which drove the relationships with defense genes. It is interesting to note that *PR1b* expression is positively correlated with, biomass (r = 0.61, P = 0.007), plant height (r = 0.67, P = 0.002) and chlorophyll content (r = 0.61, P = 0.007; Appendix B, Table B2). Whether *PR1b* expression is directly contributing to the overall better health characteristics seen in plants or, more likely, *PR1b* is simply a gene that is expressed at higher levels in healthier plants cannot readily be determined from these results or from similar results in Kumar et al. (2004) seen in the highly similar *PR1a* gene.

The available literature is extremely sparse on the effects of soil microbes on plant gene expression in tomato (and other plants in general) and is mainly limited to plant growth promoting rhizobacteria and fungi (Bent, 2006), symbionts such as mycorrhizal fungi (Taylor and Harrier, 2003), and specific soil borne pathogens (Panthee and Chen, 2010). Linkages between soil microbial community dynamics and plant gene expression have been suggested (Matoo and Abdul-Baki, 2006), but actual connections have not been made. Pearson's correlations were used to examine relationships between plant gene expression and soil microbial parameters. The FAME concentrations were not suitable for use in these correlations because relationships between concentrations and gene expression were not interpretable because they had data clustered into two distinct groups or one (or several) cases having a large leverage on the correlation coefficient.

Examining gene expression against the relative abundance of fatty acids yielded some interesting results. Expression of *ChiB* ($r = -0.69$, $P = 0.001$), *GluA* ($r = -0.49$, $P = 0.040$), and *Osm* (Figure 3.4a; $r = -0.76$, $P < 0.001$) was negatively correlated with the relative abundance of the 18:1ω7c FAME. The relative abundance of the 10Me16:0 ($r = 0.51$, $P = 0.032$) and 10:Me18:0 ($r = 0.66$, $P = 0.003$) actinomycete associated FAMEs (Zelles, 1999) and the relative abundance of the calculated sum of actinomycete FAMEs ($r = 0.49$, $P = 0.038$) were positively correlated with *ChiB* expression. Expression of the *Osm* gene was also negatively correlated with the relative abundance of the 18:3ω3 FAME ($r = -0.47$, $P = 0.048$) and the relative abundance of summed Gram negative bacterial FAMEs ($r = -0.58$, $P = 0.012$). Expression of *PR1b* was also negatively correlated to the relative abundance of summed Gram negative bacterial FAMEs (Figure 3.4b; $r = -0.63$, $P = 0.005$).

Because there is so little in the literature in this area of research, interpretation of these relationships is largely speculative. The relationship between ChiB expression and the actinomycete biomarkers is interesting in light of findings by Conn et al. (2008) who saw that actinomycetes could induce low level expression of SAR pathway related genes or jasmonate/ethylene defense pathway genes. The induction of chitinases is a component of both of these defense pathways (Busam, 1997; van Loon and Stein, 1999). Results of this study indicated that higher relative abundances of actinomycetes occurred in soils that did not receive organic amendments suggesting that if actinomycetyes play any role in modulation of plant defense gene expression that role may be more prominent when soil organic matter is at a steady state. The results of this research suggest that Gram negative bacteria may have an effect on plant defense gene expression when manure amendments are applied or in systems that regularly receive manure amendments. The 18:1ω7c FAMEs is attributed to Gram negative bacteria (Frostegård et al., 1993; D'Angelo, 2005) and the negative relationship this FAME, and the summed Gram negative bacterial FAMEs, exhibit with defense gene expression can fit with the speculation that some disease suppressive activity may be occurring with manure inputs and this suppression may be evident in the expression of defense genes. Some Gram negative bacteria, including members of the genus *Azotobacter* and members of the genus *Pseudomonas* have been shown to have antimicrobial or suppressive activity in soils, particularly against pathogenic fungi (Zegeye et al., 2011; Ponmurugan, et al. 2012; Jenifer et al., 2013). Peacock et al. (2001) found that manure amended soils favor Gram negative bacteria and suggested that *Pseudomonas* sp. are particularly competitive in manure amended soils. In addition, Mujiyati and Supriyadi (2009) observed increases in

*Azotobacter* sp. in manure amended soils. The fact that chitinase, osmotin, and β-1,3-glucanase all play roles in defense against fungal pathogens, along with the reduced expression of genes corresponding to these defense proteins in plants from manure treated and organically managed soils, would seem to support the notion of fungal pathogen suppression in these soils, possibly by a Gram negative organism such as *Azotobacter* or *Pseudomonas*. While such a scenario does not represent a direct infliuence on plant gene expression by a microorganism it does represent a scenario in which soil management effects on microbes can have effects on plant health. Activation of plant defense pathways and production of defense response proteins requires an investment of resources from the plant. If these resources are not needed for defense processes, they can be used for other resources such as building biomass or tomato fruit.

One consideration in future endeavors should be to determine the importance of gene modulation by management driven shifts in the microbial community, in the grand scheme of agroecosystems management. The scenario described above, pertaining to disease suppression and conserving plant resources warrants further investigation but the healthiest plants in this study, based on height, biomass, and chlorophyll observations (Table 3.4), were those from the vetch treatment where *PR1b* was upregulated but no suggestion of any suppressive effect is present. Assuming nutrients and water are sufficient, it remains unclear whether any one amendment would provide an additional buffer against pathogens by modulating gene expression via microbial community shifts. Given the need to understand these plant-microbe interactions more thoroughly, there is much room for future research in this arena.

In light of the results of Chapter 2 in this research, another potential area of study is the investigation of the potential for soil physical properties to affect health-related gene expression in plants. In Chapter 2 amendment driven microbial community shifts were related to changes in soil structure. It is possible that enhanced air and water movement, nutrient availabilty and root system development observed in soils with better structure could have effects on plant gene expression profiles. One problem in trying to study such effects is that it would be difficult to separate soil structure effects from amendment effects (if amendments were used to improve structure) and other soil quality-related effects, in general.

A major hurdle in research in this area is the limited resolution of microbial community assessment methods such as FAME analysis. In future studies, it is likely that researchers will need to delve beyond the microbial group level to observe conclusive connections between amendment-influenced microbial communities and plant gene responses. Newly emerging methods such as metagenomic sequencing, which provides information on functional aspects of microbial communities in addition to their composition (Fierer et al., 2012), or pyrosequencing, which yields information on the phylogenetic structure of communities (Lauber et al., 2009), may allow for advancements in this area of research.

**Conclusions**

Two plausible outcomes of the current study are evident. Firstly, modifying the soil through management practices directly influences the soil microbial community composition and induces differential expression of some genes in plants grown in

modified soil. Secondly, the changes seen in plant gene expression may, in part, be influenced by the changes in soil biology.  While differences in gene expression were observed in this study, the results with nitrogen assimilation genes and with the *ChiB*, *Osm*, and *GluA* defense genes differed from much of the limited amount of available literature, suggesting that these responses are not uniform with a given management practice and may differ among cultivars and from soil to soil.  The results also suggest that, as in *Arabidopsis thaliana* L., the *ChiB*, *Osm*, and *GluA* defense genes may be coexpressed in tomato.  The *PR1b* gene appears to be related to plant health however it is not clear whether upregulation of this gene promotes healthier plants or whether this gene is simply expressed at higher levels in healthier plants.  There is a negative relationship between expression of some defense genes and the abundance of Gram negative bacteria. Results of this research suggest that additions of manure may increase Gram negative bacteria, some of which may have disease suppressive qualities.  These disease suppressive qualities may reduce a plant's need to invest resources in induction of defense cascades and expression of defense related genes such as *ChiB*, *Osm*, *GluA*, and *PR1b*, thus allowing these resources to be used in areas such as biomass production or fruit development.   More research is needed to examine the interactions between soil management, the soil microbial community, and the genetics of plant health.

**Table 3.1.** Primers and information for the genes evaluated in this study. Presented are forward (F) and reverse (R) primers used for qPCR characterization of gene expression. Also given is each gene's GenBank accession number, primer efficiency ($E$), the amplicon size (Size) and (where applicable) a reference for the primers used in the qPCR amplification.

| Gene | Gene Product | GenBank Accession | qPCR Primers (5'-3') | $E$ | Size | Primer reference |
|------|-------------|-------------------|----------------------|-----|------|------------------|
| | | | | | bp | |
| | | | ─────Nitrogen Assimilation Genes───── | | | |
| *GS1* | Glutamine synthetase | U14754 | F: GGACCTTCTGTTGGCATCTC<br>R: GTGCTTCAAGCCAAGCTTCT | 1.97 | 225 | This study |
| *NR* | Nitrate reductase | X14060.1 | F: CCCTCTGAGGATCAAGTCTTAGG<br>R: CCCTACTTCATCGACTGTGCTAGT | 1.97 | 120 | Balaji et al., 2008 |
| *GltS* | Glutamate synthase | DB678885 | F: CCACGACCTCCTTCTGAGAG<br>R: TGGGCACACCATACATCATC | 1.91 | 160 | This study |
| *NiR* | Nitrite reductase | AW039265 | F: AGCTCGTTCCCTGAAGATCA<br>R: CGCCTTCCACAGTCTTCTTG | 1.97 | 170 | This study |
| | | | ─────Defense Response Genes───── | | | |
| *ChiB* | Basic chitinase | Z15140 | F: AACTATGGGCCATGTGGAAGA<br>R: GGCTTTGGGGATTGAGGAG | 1.92 | 128 | Song et al., 2010 |
| *Osm* | Osmotin | AY093595 | F: AGGCCAAACATGGGTCATC<br>R: CATGAACCTCTACCAGCACCA | 1.99 | 99 | Balaji et al., 2008 |
| *PR1b* | Pathogenesis-Related 1b | Y08804 | F: GCCAAGCTATAACTACGCTACCAAC<br>R: GCAAGAAATGAACCACCATCC | 1.97 | 139 | Song et al., 2010 |
| *GluA* | β-1,3-glucanase | M80604 | F: GGTCTCAACCGCGACATATT<br>R: CACAAGGGCATCGAAAGAT | 1.97 | 250 | Aime et al., 2008 |
| *PAL6* | Phenylalanine ammonia lyase | DB689083 | F: ATGCAGATCATACCCGCTCT<br>R: GGAGCACCATTCCATTCCT | 1.93 | 174 | This study |
| | | | ─────qPCR Internal Standard Genes───── | | | |
| *Act* | Actin | | F: GGAATAGCATAAGATGGCAGACG<br>R: ATACCCACCATCACACCAGTAT | 1.92 | 159 | Lovdal and Lillo, 2009 † |
| *Ubi* | Ubiquitin | X58253 | F: ACCAAGCCAAAGAAGATCAAGC<br>R: GTGAGCCCACACTTACCACAGT | 1.97 | 185 | Fiorilli et al. 2009 |

†: Based on results of the GenBank Primer-Blast tool, the second nucleotide in the *Act* forward primer was changed from A, as given in Lovdal and Lillo (2009) to a G.

**Table 3.2.** Effects of soil management treatments on chemical characteristics of a Maury silt loam. Each value represents the mean of three replicates collected 28 days post plant emergence (unless otherwise indicated). Means followed by the same letter within a row are not significantly different, as determined by the Fisher's *least significant difference* test using an $\alpha = 0.05$. Elemental concentrations were determined via Mehlich III extraction.

| Soil Characteristic | | Control | Compost | Manure | Vetch | Organic | Inorganic N |
|---|---|---|---|---|---|---|---|
| Initial pH | | $5.91^a$ | $6.14^b$ | $6.72^d$ | $6.62^c$ | $6.65^{cd}$ | $5.94^a$ |
| 28 Day pH | | $5.97^b$ | $6.18^c$ | $6.69^d$ | $5.85^a$ | $6.69^d$ | $5.85^a$ |
| Initial TotN | (%) | $0.12^a$ | $0.16^b$ | $0.17^b$ | $0.20^c$ | $0.13^a$ | $0.13^a$ |
| 28 Day TotN | (%) | $0.14^a$ | $0.18^c$ | $0.20^d$ | $0.22^e$ | $0.15^b$ | $0.19^{cd}$ |
| Initial TOC | (%) | $1.16^a$ | $1.64^c$ | $1.96^d$ | $1.74^c$ | $1.32^b$ | $1.17^a$ |
| 28 Day TOC | (%) | $1.15^a$ | $1.55^c$ | $1.79^e$ | $1.62^d$ | $1.33^b$ | $1.15^a$ |
| POXC | (mg kg$^{-1}$) | $343.84^a$ | $450.53^b$ | $521.73^c$ | $505.93^c$ | $441.50^b$ | $358.03^a$ |
| $NO_3^- -N$ | (mg kg$^{-1}$) | $2.86^a$ | $4.01^a$ | $1.87^a$ | $23.25^b$ | $1.03^a$ | $7.58^a$ |
| $NH_4^+ -N$ | (mg kg$^{-1}$) | $2.99^a$ | $4.10^a$ | $12.94^d$ | $10.24^c$ | $3.08^a$ | $6.25^b$ |
| P | (mg kg$^{-1}$) | $35.73^a$ | $72.61^c$ | $102.09^d$ | $53.02^b$ | $116.08^e$ | $35.07^a$ |
| K | (mg kg$^{-1}$) | $170^a$ | $339^c$ | $328^c$ | $714^d$ | $293^b$ | $169^a$ |
| Ca | (mg kg$^{-1}$) | $1765^{bc}$ | $2139^d$ | $1875^c$ | $1710^b$ | $2650^e$ | $1453^a$ |
| Mg | (mg kg$^{-1}$) | $198^b$ | $254^c$ | $332^e$ | $213^b$ | $272^d$ | $172^a$ |
| Zn | (mg kg$^{-1}$) | $1.66^a$ | $4.59^b$ | $10.59^c$ | $2.60^a$ | $3.97^b$ | $1.73^a$ |
| Cu | (mg kg$^{-1}$) | $2.52^b$ | $2.84^c$ | $2.74^c$ | $2.26^a$ | $2.15^a$ | $2.59^b$ |
| Mn | (mg kg$^{-1}$) | $219^b$ | $198^a$ | $198^a$ | $220^b$ | $239^c$ | $231^c$ |
| Na | (mg kg$^{-1}$) | $10.90^a$ | $44.03^c$ | $105.74^d$ | $13.04^a$ | $8.30^a$ | $27.46^b$ |
| Fe | (mg kg$^{-1}$) | $103^a$ | $113^c$ | $104^a$ | $104^a$ | $132^d$ | $108^b$ |
| Ni | (mg kg$^{-1}$) | $0.38^a$ | $0.76^a$ | $1.25^b$ | $0.66^a$ | $0.34^a$ | $0.61^a$ |
| Al | (mg kg$^{-1}$) | $1031^d$ | $969^b$ | $935^a$ | $963^b$ | $1017^d$ | $996^c$ |
| CEC | (cmol$_c$ kg$^{-1}$) | $13.55^a$ | $16.72^c$ | $15.25^{bc}$ | $15.20^b$ | $14.78^{ab}$ | $14.01^{ab}$ |
| Base Sat. | (cmol$_c$ kg$^{-1}$) | $74.30^{ab}$ | $76.49^{ab}$ | $88.24^c$ | $82.47^{bc}$ | $89.96^c$ | $67.25^a$ |
| Exch. K | (cmol$_c$ kg$^{-1}$) | $0.43^a$ | $0.84^c$ | $0.86^c$ | $2.15^d$ | $0.70^b$ | $0.44^a$ |
| Exch. Ca | (cmol$_c$ kg$^{-1}$) | $7.91^b$ | $9.82^e$ | $9.31^d$ | $8.41^c$ | $10.79^f$ | $7.46^a$ |
| Exch. Mg | (cmol$_c$ kg$^{-1}$) | $1.42^b$ | $1.86^d$ | $2.58^e$ | $1.66^c$ | $1.72^c$ | $1.32^a$ |
| Exch. Na | (cmol$_c$ kg$^{-1}$) | $0.22^{ab}$ | $0.27^b$ | $0.68^c$ | $0.30^b$ | $0.07^a$ | $0.21^{ab}$ |

**Table 3.3**. Effects of soil management treatments on concentrations and relative abundances of soil fatty acid methyl ester (FAME) biomarkers after 28 days of tomato growth. Effects on fungal ergosterol concentration are also presented. Concentrations and relative abundances are presented for arbuscular mycorrhizal fungi (AMF), actinomycetes (Actino), fungi, bacteria, Gram positive bacteria (Gram+), Gram negative bacteria (Gram −), and total FAMEs. Total FAMEs includes other FAMES (eukaryotic FAMEs and FAMEs not designated as signature biomarkers). Also presented is the ratio of fungal FAME to bacterial FAMEs (F:B). Effects on the relative abundances of individual FAME biomarkers are presented next to or beneath the microbial group with which they are associated. Each value represents the mean of three replicates. Means in the same row are not significantly different when followed by the same letter, as determined by Fisher's least significant difference test at $\alpha = 0.05$.

| | units | Control | Compost | Manure | Vetch | Organic | Inorganic N |
|---|---|---|---|---|---|---|---|
| | | | | Soil Management Treatment | | | |
| Microbial Group | | | | Concentrations | | | |
| AMF | (µmol kg$^{-1}$) | 6.78$^a$ | 15.08$^b$ | 14.19$^b$ | 21.40$^c$ | 8.78$^a$ | 5.24$^a$ |
| Actino | (µmol kg$^{-1}$) | 6.68$^{ab}$ | 12.77$^{cd}$ | 22.34$^e$ | 15.98$^d$ | 10.25$^{bc}$ | 5.84$^a$ |
| Fungi | (µmol kg$^{-1}$) | 10.60$^a$ | 15.52$^a$ | 133.42$^c$ | 76.67$^b$ | 15.48$^a$ | 8.44$^a$ |
| Bacteria | (µmol kg$^{-1}$) | 46.05$^a$ | 87.79$^b$ | 139.95$^d$ | 109.07$^c$ | 71.67$^b$ | 31.39$^a$ |
| Gram + | (µmol kg$^{-1}$) | 12.35$^a$ | 26.86$^c$ | 39.32$^e$ | 33.24$^d$ | 21.70$^b$ | 9.97$^a$ |
| Gram − | (µmol kg$^{-1}$) | 8.48$^{ab}$ | 15.17$^b$ | 35.41$^d$ | 23.99$^c$ | 14.90$^b$ | 5.69$^a$ |
| Total FAMEs | (µmol kg$^{-1}$) | 144.75$^a$ | 324.40$^c$ | 621.57$^e$ | 474.52$^d$ | 235.66$^b$ | 110.83$^a$ |
| Ergosterol (fungi) | (mg kg$^{-1}$) | 0.60$^a$ | 0.84$^{ab}$ | 2.00$^c$ | 2.50$^d$ | 1.25$^b$ | 0.50$^a$ |
| | | | | Ratio of Fungal FAME to Bacterial Fames | | | |
| F:B | | 0.23$^a$ | 0.19$^a$ | 0.95$^c$ | 0.70$^b$ | 0.22$^a$ | 0.27$^a$ |
| | | | | Relative Abundances (%) | | | |
| AMF: 16:1 ω5c | (%) | 4.59 | 4.64 | 2.30 | 4.57 | 3.73 | 4.72 |
| Actino | (%) | 4.76$^{bc}$ | 3.94$^{ab}$ | 3.58$^{ab}$ | 3.37$^a$ | 4.36$^b$ | 5.34$^c$ |
| 10me16:0 | (%) | 3.29$^{cd}$ | 2.64$^{abc}$ | 2.47$^{ab}$ | 2.15$^a$ | 2.93$^{bc}$ | 3.79$^d$ |
| 10me17:0 | (%) | 0.37 | 0.16 | 0.31 | 0.17 | 0.26 | 0.24 |
| 10me18:0 | (%) | 1.10$^b$ | 1.14$^b$ | 0.80$^a$ | 1.05$^b$ | 1.17$^b$ | 1.32$^c$ |
| Fungi : 18:2ω6c | (%) | 7.35$^b$ | 5.09$^a$ | 21.57$^d$ | 16.07$^c$ | 6.60$^{ab}$ | 7.57$^b$ |
| Bacteria† | (%) | 31.64$^d$ | 27.08$^b$ | 22.63$^a$ | 23.02$^a$ | 30.36$^{cd}$ | 28.50$^{bc}$ |
| 14:0 | (%) | 1.67$^b$ | 1.93$^c$ | 1.26$^a$ | 1.13$^a$ | 1.78$^b$ | 1.74$^b$ |
| a15:0 | (%) | 3.15$^{ab}$ | 2.55$^a$ | 2.67$^a$ | 2.91$^{ab}$ | 3.53$^b$ | 3.24$^{ab}$ |
| 15:0 | (%) | 0.69 | 0.66 | 0.58 | 0.61 | 0.69 | 0.72 |
| i17:0 | (%) | 1.83$^b$ | 2.20$^c$ | 1.31$^a$ | 1.32$^a$ | 1.92$^b$ | 1.79$^b$ |

**Table 3.3**. (Continued)

| | units | Control | Compost | Manure | Vetch | Organic | Inorganic N |
|---|---|---|---|---|---|---|---|
| | | | | Soil Management Treatment | | | |
| Bacteria† (cont.) | | | | Relative Abundances (%) | | | |
| a17:0 | (%) | 2.05[b] | 2.65[c] | 1.39[a] | 1.63[a] | 2.23[b] | 2.02[b] |
| 16:1ω7c | (%) | 5.64[d] | 3.00[a] | 3.37[ab] | 3.02[a] | 4.53[c] | 4.33[bc] |
| i17:1ω7c | (%) | 1.04[ab] | 0.88[a] | 0.87[a] | 0.84[a] | 1.28[b] | 0.98[a] |
| cy19:0 | (%) | 1.91[b] | 3.06[c] | 1.26[a] | 1.46[a] | 1.92[b] | 2.15[b] |
| Gram + | (%) | 8.67[c] | 8.28[bc] | 6.40[a] | 7.01[ab] | 9.25[c] | 9.11[c] |
| i15:0 | (%) | 6.01[b] | 4.77[a] | 4.54[a] | 4.79[a] | 6.61[b] | 6.51[b] |
| i16:0 | (%) | 2.66[b] | 3.51[c] | 1.86[a] | 2.23[ab] | 2.65[b] | 2.60[b] |
| Gram – | (%) | 5.80[bc] | 4.68[a] | 5.65[bc] | 5.07[ab] | 6.28[c] | 5.14[ab] |
| cy17:0 | (%) | 1.20[c] | 0.40[a] | 0.83[b] | 1.00[bc] | 1.14[bc] | 1.29[c] |
| 18:1ω7c | (%) | 4.23[ab] | 4.28[ab] | 4.83[bc] | 4.07[a] | 5.15[c] | 3.86[a] |
| Additional FAMEs‡ | | | | | | | |
| 18:3ω3 | (%) | 0.64[ab] | 0.45[ab] | 0.27[a] | 1.04[c] | 0.72[bc] | 0.58[ab] |
| 18:1ω9c | (%) | 9.67 | 9.36 | 12.46 | 8.80 | 8.42 | 9.75 |
| 18:1ω5c | (%) | 2.49[b] | 3.15[c] | 4.39[d] | 9.33[e] | 3.30[c] | 1.85[a] |
| 20:4ω6 | (%) | 0.45[b] | 1.54[d] | 0.00[a] | 0.42[b] | 0.81[c] | 0.54[bc] |
| 20:5ω3 | (%) | 0.88 | 0.40 | 1.10 | 0.83 | 0.66 | 0.92 |

†: Includes Gram + and Gram – FAMEs

‡: FAMEs of microbial origin that have been associated with more than one microbial group in the literature.

**Table 3.4.** Effects of soil management treatments on tomato plant characteristics. Each value represents the mean of three replicates collected 28 days post plant emergence. Means in the same row are not significantly different when followed by the same letter, as determined by the Fisher's least significant difference test using an $\alpha = 0.05$.

| Plant Characteristic | units | Soil Management Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | Compost | Manure | Vetch | Organic | Nitrogen |
| Plant Height | (cm) | $20.67^a$ | $29.87^c$ | $26.30^{bc}$ | $30.13^c$ | $20.87^a$ | $24.53^{ab}$ |
| Dry Biomass | (g) | $1.16^a$ | $5.49^b$ | $4.99^b$ | $8.92^c$ | $1.71^a$ | $2.22^a$ |
| Chlorophyll | (g kg$^{-1}$) | $1.47^{ab}$ | $1.65^b$ | $1.55^{ab}$ | $2.07^c$ | $1.23^a$ | $1.70^b$ |
| Macronutrients | | | | | | | |
| Ca | (g kg$^{-1}$) | $21.03^b$ | $16.13^a$ | $16.08^a$ | $21.60^b$ | $23.19^b$ | $20.24^b$ |
| K | (g kg$^{-1}$) | $22.33^{ab}$ | $18.74^a$ | $19.31^{ab}$ | $26.92^c$ | $20.98^{ab}$ | $23.25b^c$ |
| Mg | (g kg$^{-1}$) | 5.88 | 4.54 | 5.2 | 5.08 | 5.71 | 5.84 |
| P | (g kg$^{-1}$) | $3.89^b$ | $2.44^a$ | $3.87^b$ | $2.09^a$ | $5.28^c$ | $2.40^a$ |
| Kjeldahl N | (g kg$^{-1}$) | $9.32^a$ | $8.32^a$ | $7.91^a$ | $12.17^b$ | $8.39^a$ | $21.31^c$ |
| Micronutrients | | | | | | | |
| Cd | (mg kg$^{-1}$) | $0.26^{bc}$ | $0.16^{ab}$ | $0.09^a$ | $0.27^c$ | $0.22^{bc}$ | $0.25^{bc}$ |
| Co | (mg kg$^{-1}$) | 0.24 | 0.17 | 0.14 | 2.13 | 0.31 | 0.66 |
| Cr | (mg kg$^{-1}$) | $1.98^{ab}$ | $1.79^{ab}$ | $0.88^a$ | $1.07^{ab}$ | $2.63^{bc}$ | $3.79^c$ |
| Cu | (mg kg$^{-1}$) | 39.68 | 26.52 | 23.67 | 13.58 | 30.85 | 20.45 |
| Fe | (mg kg$^{-1}$) | 225.96 | 78.91 | 56.24 | 70.03 | 77.7 | 227.77 |
| Mn | (mg kg$^{-1}$) | $96.19^{abc}$ | $86.62^{ab}$ | $47.78^a$ | $182.16^c$ | $53.53^a$ | $169.83^{bc}$ |
| Mo | (mg kg$^{-1}$) | $1.24^{ab}$ | $0.47^a$ | $2.60^b$ | $0.22^a$ | $6.34^c$ | $0.65^a$ |
| Ni | (mg kg$^{-1}$) | $1.09^a$ | $0.46^a$ | $0.43^a$ | $0.58^a$ | $0.90^a$ | $1.95^b$ |
| Zn | (mg kg$^{-1}$) | 74.86 | 46.2 | 43.87 | 51.44 | 52.62 | 48.9 |

**Table 3.5**. Chemical characteristics of the three organic amendment materials used in the study. Total organic carbon (TOC) and total nitrogen were determined by dry combustion., the amendment C fractions were determined using a stepwise proximal fractionation, and the elemental analysis was determined by a nitric acid digestion followed by inductively coupled plasma spectroscopy.

| Amendment Characteristic | units | Compost | Amendment Manure | Vetch |
|---|---|---|---|---|
| TOC | (%) | 17.4 | 43.9 | 40.9 |
| Nonpolar C | (% of TOC) | 2.2 | 3.9 | 3.2 |
| Water Soluble C | (% of TOC) | 7.1 | 11.4 | 24.9 |
| Acid Soluble C | (% of TOC) | 17.1 | 21.3 | 29 |
| Lignin & Humic C | (% of TOC) | 73.6 | 63.4 | 42.9 |
| Total N | (%) | 1.4 | 2.6 | 4.0 |
| C:N Ratio | | 12.4 | 16.9 | 10.2 |
| Ca | $(g\ kg^{-1})$ | 20.0 | 30.3 | 9.9 |
| K | $(g\ kg^{-1})$ | 6.7 | 11.7 | 28. 9 |
| Mg | $(g\ kg^{-1})$ | 5.2 | 10.4 | 2.4 |
| P | $(g\ kg^{-1})$ | 3.1 | 9.5 | 4.2 |
| Co | $(mg\ kg^{-1})$ | 10.0 | 2.5 | 0.4 |
| Cr | $(mg\ kg^{-1})$ | 27.0 | 2.9 | 0.7 |
| Cu | $(mg\ kg^{-1})$ | 62.2 | 136.4 | 13.9 |
| Fe | $(mg\ kg^{-1})$ | 11800 | 1240 | 303.5 |
| Mn | $(mg\ kg^{-1})$ | 363.1 | 248.3 | 90.8 |
| Mo | $(mg\ kg^{-1})$ | 1.0 | 3.9 | 0.4 |
| Ni | $(mg\ kg^{-1})$ | 26.4 | 7.9 | 1.5 |
| Zn | $(mg\ kg^{-1})$ | 176.6 | 1010 | 71.2 |

**Figure 3.1.** Effects of soil management on the expression of selected nitrogen assimilation (3.1a-3.1d) and defense related (3.1e-3.1i) genes. Each bar represents the mean of the $\log_2$ transformed relative expression data (generated using the $2^{-\Delta\Delta C_T}$ method) from three replications. For each gene, values are not significantly different, as determined by Fisher's least significant difference at an $\alpha = 0.05$, when bars representing treatment means share the same letter.

**Figure 3.2.** Linear regression showing the relationship between total FAMEs and (a) total soil organic C and (b) permanganate oxidizable C (POXC). Each coordinate within a plot represents one biological replicate for a given soil management treatment and *** indicates that the relationship is significant at $P < 0.001$.

**Figure 3.3**. (a) Non-metric multidimensional scaling (NMS) ordination of FAME biomarkers, based on relative abundance, after 28 days of tomato growth. This FAME ordination determines the ordination of treatment-associated microbial communities in 3.3b. Axes in 3.3a and 3.3b correspond to each other. (b) NMS joint plot showing relationships between treatment-associated soil microbial communities and soil properties after 28 days of tomato growth. Soil properties include Mehlich III extractable elements (Al, K, Mg, Na, Ni, Zn), total organic C (TOC), permanganate oxidizable C (POXC), $NO_3^-$–N, $NH_4^+$–N, total N, base saturation, and CEC. Also included in 3.3b are vectors that represent microbial biomass (total FAMEs), ergosterol, and the relative abundances of actinomycetes, arbuscular mycorrhizal fungi (mycorrhizae), bacteria, Gram negative bacteria (Gram –), and fungi. The box at the lower left is a magnification of the smaller box above it to allow for labeling of the biomarkers.

**Figure 3.4.** Pearson's correlations showing relationships between (a) the relative abundance of the 18:1ω7c Gram negative bacteria associated FAME biomarker and Log2 transformed relative expression of the Osm defense gene, and (b) the relative abundance of summed Gram negative bacteria associated FAME biomarkers and Log2 transformed relative expression of the PR1b defense gene. Each point is one experimental replicate. The Guassian bivariate confidence ellipses have P = 0.63 and ** or *** indicate significance at P < 0.01 or P < 0.001, respectively.

# CHAPTER 4

## CONCLUSIONS, IMPLICATIONS, AND FUTURE DIRECTIONS

**Research Findings and Implications**

The linkages between soil management with organic amendments and changes in soil chemical and microbial properties and plant responses discovered in this study are summarized in Figure 4.1.

The experiment conducted in Chapter 2 systematically compared the effects of three organic amendments with divergent organic C bioavailabilities on microbial community composition and development of soil structure in three soils with different soil physical and chemical properties. The specific research question was "*Can organic amendments be used to develop a more fungal dominated community that fosters improved soil structure*?" Results from the study showed that certain organic amendments (vetch and manure) rapidly stimulated fungal proliferation in the soils, as indicated by increased levels of 18:2ω6c and ergosterol fungal biomarkers. Furthermore, fungal proliferation was linked to the rapid formation of large macroaggregates in the vetch- and manure-amended soils. In contrast, vegetable compost had little effect on fungal proliferation or large macroaggregate formation in any of the soils. These differences were attributed to variations in bioavailable organic carbon in the amendments, as indicated by proximate organic carbon fractionation and biodegradability of organic C. Results provided conclusive evidence that soils can be managed to maintain or improve soil structure via amendment with organic materials that promote fungal proliferation by providing high levels of labile and semi-labile organic C. The

results have important implications on agricultural production, due to the ecosystem services (soil C sequestration, air and water movement, reduced erosion, improved nutrient availability, well developed plant root systems, seedling emergence) provided in a well structured soil.

In Chapter 3 an experiment was conducted to determine whether individual organic amendments with divergent organic C bioavailabilities, or a management system (organic management) that historically has used a combination of these amendments, affected microbial community composition and expression of genes important to nitrogen assimilation and plant defense against responses. Specific research questions were "*Do organic amendments have significant effects on expression of nitrogen assimilation genes and defense response genes by plants, and how are differences related to changes in soil chemical and microbiological properties due to the amendments?*" It was discovered that organic amendments affected expression of a nitrogen assimilation gene and several defense-related genes in tomato. Specifically, expression of the pathogenesis-related *PR1b* gene (which codes for the pathogenesis-related PR1b protein) was increased in plants grown in soil amended with compost, vetch, and N fertilizer, while the expression of three other defense-related genes that code for chitinase (*ChiB*), osmotin (*Osm*), and β-1,3-glucanase (*GluA*) were decreased in plants grown in soil that was amended with manure and in plants grown in soil that had been managed with organic production practices. Furthermore, expression of the nitrogen assimilation gene *GS1*, which codes for glutamine synthetase, was lower in plants grown in soils treated with inorganic N fertilizer.

It was difficult to pinpoint specific mechanisms for the changes in gene expression. Reduced expression of the GS1 gene in inorganic N fertilized could not be conclusively linked to a specific soil chemical or microbial property. The differential expression of defense related genes was inversely related to the relative abundance of Gram negative bacteria. The relative abundance of the 18:1ω7c Gram negative bacterial biomarker was highest in manure treated soil and organically managed soil (which has a history of receiving manure amendments). These treatments also generally had the lowest expression of *ChiB*, *Osm*, and *GluA* leading to speculation that manure amendment, through increases in Gram negative bacteria, may have suppressed populations of soil organisms that induce a defense response in plants, possibly allowing for less stressed plants. This research has important implications on agriculture in that is shows that different amendments or management schemes can affect health related gene expression in crops. It also raises questions about the mechanisms behind these changes in gene expression, providing ample fodder for future research.

**Recommendations for Future Research**

Results from this study showed that certain types of organic amendments affected the formation of soil macroaggreates, due primarily to their influence on the soil fungal community. While efforts were made to evaluate amendments and soils with wide ranging characteristics, it would be interesting in future studies to determine the validity of study conclusions by evaluating different soils, amendments, and combinations of amendments that are commonly used in agricultural production systems. Furthermore, conclusions presented here were based on laboratory studies of short duration (a few

months), so it is suggested that future projects determine the validity of conclusions at the field scale and over the long term.

The primary focus of research in Chapter 2 was on amendment effects on formation of macroaggregates in soils, but amendments could also influence agricultural sustainability in other ways that were not investigated in this study. For example, it is possible that increased soil aggregation by the addition of amendments could, via macropore flow, increase water and nutrient mobility and losses, particularly if amendments are rich in nutrients (e.g. manure), or are applied outside of the growing season. Future studies should be conducted to determine the role of amendments on these and other processes that could impact agricultural sustainability.

Research in Chapter 3 leaves much room for further and expanded study. To my knowledge, there has been little research comparing the effects of various soil management practices on gene expression in plants. While this study begins to fill that gap, the results of this work cannot be considered definitive. Additional studies should be conducted with soils having diverse physical properties and chemical attributes. Gene expression in other plants should be conducted to determine whether the relationships found in this study are widely applicable to other plants and soils. Certainly, additional genes should be examined, perhaps using more sophisticated techniques such as microarray, which has been used to show the effects of agricultural management on gene expression in other studies (Tenea et al. 2012). Establishment of relationships between specific agricultural management practices and gene expression are critical to farmers that need to optimize conditions in their soils for plant health and productivity.

A limitation of research described here is low resolution of the fatty acid methyl ester analysis to examine the specific members of the microbial community involved in soil and plant improvement activities.  New techniques are emerging that may allow researchers to more closely examine the phylogeny and function of particular members of the microbial community involved in key processes in variously managed soils.  Among the methods that may be useful in future research are shotgun metagenomic sequencing and pyrosequencying (Tringe et al., 2005; Lauber et al., 2009; Fierer et al., 2012).  Such knowledge could provide scientists and producers with more precise and scientifically valid strategies for managing soil microbial communities in order to enhance plant health.  Eventually, knowledge could be integrated into models and overarching management plans in order to maximize soil quality and plant health in sustainable agroecosystems.

**Figure 4.1.** Conceptual diagram summarizing the findings discovered in this study (solid lines) and future directions (dashed lines).

# APPENDIX A

## A.1: SUPPLEMENTAL INFORMATION ON TOMATO (*SOLANUM LYCOPERSICUM*) GENES INVESTIGATED IN CHAPTER 3

*Defense Response Genes*

**<u>ChiB</u>:** Basic 30 kDa endochitinase precursor - *Solanum lycopersicum* (Tomato)
- 69% similar to Arabidopsis HCHIB (At3g12500: Basic chitinase).   Chitinase activity, Endochitinase activity, Defense response to fungus, Cell wall catabolic process, Chitin catabolic process, Response to other organism.
- Role in plant: Defense response.
- Potentially coexpressed with: A-DOX1, OSM, PR2
- Reference: Kumar et al. (2004)
- Primer reference: Song et al. (2010)
  - F (5'-3'): AACTATGGGCCATGTGGAAGA
  - R (5'-3'): GGCTTTGGGGATTGAGGAG
  - Product Length: 128bp

<u>DFCI – Tomato TC Report*:</u> TC219426
<u>GenBank accession number:</u> Z15140

<u>Nucleotide sequence:</u>
ATCGGCCGAATTGATCAACTAATTTTACTAATACATTAAAAAAAAATGAGGCTTTCT
GAATTCACTACTCTTTTCTTACTATTTTCTGTGCTTTTGCTGTCTGCCTCTGCAGAGCA
ATGTGGTTCACAGGCCGGAGGCGCACTTTGTGCATCCGGACTGTGTTGCAGTAAATT
TGGTTGGTGTGGTAACACTAATGAGTATTGTGGTCCTGGTAATTGTCAGAGCCAGTG
TCCTGGCGGTCCCGGTCCTTCAGGGGACCTAGGCGGTGTTATTTCAAATTCCATGTTT
GATCAAATGCTTAATCATCGCAATGACAATGCTTGTCAAGGAAAGAATAATTTCTAC
AGTTACAATGCATTTGTTACTGCTGCTGGGTCTTTTCCTGGATTTGGTACTACTGGGG
ATATCACTGCCCGTAAAAGGGAAATTGCTGCTTTCCTTGCCCAAACTTCCCATGAAA
CTACTGGAGGATGGCCTACGGCACCAGATGGACCATACGCATGGGGTTACTGTTTCC
TTAGAGAGCAAGGTAGCCCTGGCGATTACTGTACACCAAGTAGTCAATGGCCTTGTG
CTCCTGGAAGGAAATATTTCGGACGAGGTCCAATTCAAATTTCACACAACTACAACT
ATGGGCCATGTGGAAGAGCCATTGGAGTGGACCTTTTGAACAATCCCGATCTAGTAG
CAACAGACCCAGTCATCTCATTCAAATCAGCTATCTGGTTCTGGATGACTCCTCAATC
CCCAAAGCCTTCTTGTCACGATGTCATCACCGGAAGATGGCAGCCATCTGGCGCTGA
CCAAGCAGCTAATCGCGTCCCTGGATTCGGTGTCATCACAAACATCATCAATGGTGG
CCTGGAATGTGGTCACGGCAGTGACAGCAGGGTCCAGGATCGGATTGGATTTTACAG
GAGGTATTGCGGAATTCTTGGAGTTAGCCCAGGTGAAAATCTTGATTGTGGCAATCA
GAGGTCTTTTGGAAACGGACTATTAGTTGATATTATGTAACGATCGACTTCATCATAT
AAGGCCCCAACTATAAATAAATTTAATATGTATGAAATTGTATGAATTATGATTGTA
ATATGATGGGACCTTGTCTTATAATCGTTTGTTTTGATAATAAACAAAGACTACGATG
GTTCTTATTCTAATTAATGTGGTGACGGGTCATATGTTAATTAGCATTATGGAAGCTT
ATTAACAATCGAGCAATTTCATTACATTGTTTAGG

*GluA***:** beta-1,3-glucanase - *Solanum lycopersicum* (Tomato)
55% similar to Arabidopsis ATBG3  (AT3G57240.1
- (beta-1,3-glucanse).
- Role in plant: Pathogenesis related protein, MAPK cascade, carbohydrate metabolic process, defense response to bacterium, defense response to fungus, defense response, incompatible interaction, detection of biotic stimulus, jasmonic acid mediated signaling pathway, negative regulation of defense response,.
- Primer Reference: Aime et al. (2008)
  - F (5' -3'): GGTCTCAACCGCGACATATT
  - R (5' – 3'): CACAAGGGCATCGAAAAGAT
  - Product length 250bp

DFCI – Tomato TC Report*: TC222966
GenBank accession number:  M80604

 Nucleotide sequence:
TCAATTTCCAATTTTGCTATGGCTTTTCTAAGTTCTCTCTTAGCTTCCCTTTTACTTGTT
GGGCTTCTAATCCAAATAACAGGAGCGCAGCCTATCGGAGTATGTTATGGAAAAATT
GCCAATAATTTACCATCGGATCAAGATGTCATAAAATTATATAATTCGAATAACATC
AAGAAAATGAGAATTTACTTTCCAGAAACAAATGTCTTTAATGCCCTCAAAGGAAGT
AACATTGAAATAATTCTTGATGTCCCAAATCAAGATCTTGAAGCCCTAGCCAATCCTT
CCAAACGCCAAGGTTGGGTTCAAGATAATATAAGAAATCACTTTCCGGATGTTAAAT
TCAAATATATAGCCGTTGGAAACGAAGTTGATCCAGGTAGAGACAGTGGTAAATAC
GCACGATTTGTTGGTCCAGCAATGGAAAATATTTACAACGCGTTATCATCAGCAGGG
TTGCAAAATCAAATCAAGGTCTCAACCGCGACATATTTAGGGCTTTTAACCAACACC
TACCCACCTAGAGATAGCATTTTTCGCGATGAATATAAAAGTTTCATCAATCCCATA
ATTGGATTTCTATCAAGACATAATCTTCCACTTTTAGCCAATATTTACCCTTATTTTGG
CCATGCTGATGATAATGTTCCTCTTCCTTATGCACTTTTCAAGCAACAAGGGCTAAAC
GATGCAGGATATCAAAATCTTTTCGATGCCCTTGTGGATTCAATGTATTTTGCTACTG
AGAAACTTGGAGGACAAAATATTGAGATTATTGTATCGGAAAGTGGTTGGCCTTCTG
AAGGACACCCTTCCGCTACTCTTGAAAACGCGATGACTTATTATACAAACTTGATTA
ATCATGTGAAAGGAGGGGCAGGAACACCAAAGAAACCAGGAAGGACTATAGAAAC
CTATTTATTCGCCATGTTTGATGAAAATAGAAAGGATGGAAAACCAAGTGAGCAACA
TTTTGGACTCTTTAAACCTGACCAGAGGCCAAAGTATCAACTCAAATTTGATTAATG
ATACCATATTATAATCAATATATAGATATTTTATGTTATGTATATGAATAATCAATAA
ATGATAGTATATGTGAGAAAAATGTATGGCCTCTTTTGTGTTCCTTGATATCATAACA
TTAATATTGAAGTATTTTTAATAAGATAGGTATTTTAATTGA

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***Osm*:** Osmotin-like protein – (PR5x) *Solanum lycopersicum* (Tomato)
- 63% similar to Aprabidopsis OSM34 (At4g11650: Osmotin). Defense response, expressed in root.
- Role in plant: Defense response.
- Potentially coexpressed with: A-DOX1, chiB, PR2
- Reference: Kumar et al. (2004)
- Primer Reference: Balaji et al. (2008)
  - F (5'-3'): AGGCCAAACATGGGTCATC
  - R (5'-3'): CATGAACCTCTACCAGCACCA
  - Product length: 99bp

DFCI – Tomato TC Report*: TC218496; TC193267
GenBank accession number: AY093595

Nucleotide sequence:
ATCGGCCGAATTGAACAACTTACATTTAAAAAAATAGTTCCACAAACATGGCCTACT
TGAGATCTTCTTTTGTTTTCTTCCTTCTTGCTTTTGTGACTTACACTTATGCTGCCACTT
TCGAGGTACGCAACAACTGTCCATACACCGTCTGGGCGGCGTCGACCCCAATAGGCG
GTGGTCGACGTCTTGATCGAGGCCAAACATGGGTCATCAATGCACCGAGGGGCACTA
AGATGGCACGTATATGGGGTCGTACGAATTGCAACTTTGATGGTGCTGGTAGAGGTT
CATGTCAGACTGGTGATTGTGGTGGGGTCTTGCAATGTACCGGGTGGGGCAAACCAC
CAAACACCCTGGCCGAGTACGCCTTGGACCAGTTTAGCAACCTAGATTTCTGGGACA
TTTCTTTAGTCGATGGATTTAATATTCCAATGACTTTCGCCCCGACCAATCCTAGTGG
AGGGAAATGCCATGCAATTCATTGTACGGCTAATATAAATGGTGAATGTCCTGGTTC
ACTTAGGGTACCCGGAGGATGTAACAATCCTTGTACCACGTTCGGAGGACAACAATA
TTGTTGCACACAAGGTCCATGTGGCCCTACTGATTTGTCGAGATTTTTCAAACAAAGA
TGTCCTGATGCGTATAGCTACCCACAAGATGATCCTACTAGCACATTTACTTGCCCTA
GTGGTAGTACAAATTATAGGGTTGTTTTTTGTCCTAATGGTGTTACTAGCCCAAATTT
CCCCTTGGAGATGCCCTCAAGTGATGAAGAGGCTAAGTAAAATTGAGTCACTTTCTT
TTAAATTGCTTGAAGTAGTCGAGTTATATAATTGGCTTGTAATAAACCTAATATAATT
ACATGAATAAAAGTCACATCATCACAAATATGTTGTTTCGAATATTATTTTATGTATA
TTTTGATATTTACGTATTAACT

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***PAL6***: phenylalanine ammonia lyase 6- *Solanum lycopersicum* (Tomato)

- General stress and defense response
- Reference: Gayoso et al. (2010)
- Primers Designed with Primer 3 Plus
    - F (5' -3'): ATGCAGATCATACCCGCTCT
    - R (5' – 3'): GGAGCACCATTCCATTCCT
    - Product length 174

DFCI – Tomato TC Report*: TC234798
GenBank accession number:  DB689083

 Nucleotide sequence:
GCTCAATCTTTCAAAGGATTGTAGCTTATAAGAAGAGTTGAAGTCTGTTTTACCAAG
AGAAGTCGAGAGTGCCAGAGTCGCGTTGGAAAGTGGAAACCCCGCGATTGCAAACA
GGATCAACGAATGCAGATCATACCCGCTCTACAAGTTTGTTAGGGAGGAGCTCGGGA
CGGAATTGTTGACAGGAGAAAGAGTAAGATCACCAGGTGAAGAATGTGACAAGGTG
TTCACAGCAATGTGCAATGGACAAATCATTGATTCATTGTTAGAATGCCTTAAGGAA
TGGAATGGTGCTCCACTACCAGTCTGTTAGAAGTGAAGCAACAGGTTTTATTATGTT
AAATGTTTGTCAATTACTTCAATTATTTTTACATTTACCATTTTGGAGTTAAAAACTA
AATATGAACTCTCTTGAATATATTGGTTTGTAGCTATATATTATTACAAGTCCTTTTTT
ATTCTATGAAAATAAAAGAAAAGTGACCTTGTGCTTAT

**_PR1b:_** Pathogenesis-related leaf protein 6 precursor(aka: PR1, PR1b, PR1b1, PR6) - _Solanum lycopersicum_ (Tomato)

- 60% similar to Arabidopsis ATPR1  (>AT2G14610.1: pathogenesis-related gene 1).
- Response to ethylene stimulus, response to jasmonic acid stimulus, response to salicylic acid stimulus; defense response
- Role in plant: Systemic acquired resistance
- Potentially coexpressed with: LeMKK2; MPK3; MPK1
- Reference: Xing et al. (2001); Tornero et al. (1997); Block et al. (2005)
- Primer reference: Song et al. (2010)
    - F (5'-3'): GCCAAGCTATAACTACGCTACCAAC
    - R (5'-3'): GCAAGAAATGAACCACCATCC
    - Product Length: 139bp

DFCI – Tomato TC Report*: TC218002
GenBank accession number:  Y08804


Nucleotide sequence:
CATATCAAACTATTTATGCTATATTTAATACTTATTTAAATCGTAACTATTTATTTCTA
AATTGCAGCTCTAACACTTTTTAAAAAGTTTTTAAAATTTTCTTTCGAAGCTACAAGG
ATTGAGGCAGTTTCTAATTTCCACATAAAGGACCAAAAAATTGTTTTGGTTTAAGTGT
GTCTATCACTTCCATTGTTTGTTGTTTTCATTATATCCATTATATCCAAATTGCTTTCA
ATGACTAACAAATACTTGAGTCTTCTCTCTCTCATAGAATAACTTTCTTCCATAAATC
CACGTAAGGCGGCTCAATAAGTGTTGTTTAATATTATCTAAATAATAGAGTAAAGTA
TGATTATTCTTTAAAGCATATAGTATTTCCTTAATCACACGACATGCAATCTCCTTTG
AATTTTCTTCACATGTGAATAAAATTCCACAAAGTTTAAATTTAACAATTTTCACAAA
TTTGATTAAATTGAACAATCAAAGTTTGATAAATATCTTATCGAAACCAAAAGTGTT
CACTTTTGAAAAATATAAAGGACCAAAAAAGATCACTTTTGACAATCTTAAGGACCA
AAAATTGCTCTTATCATACATAAGGGAGTCTATTGAAACTACTAAAAAGATAAGGAAG
CCTTTTTGTCATTTCTCTATAATTTCCTTTGAATTTTTCTTCATACATGCTAAACAAAA
TTACACAATGTTTAATTATATATACACTATTTAAAGGACCAAAAGTAATTAATTCTAA
AAAGTTTTAAGGACCAAAATCACTTAGCAAAATACACAAGAGACTATTTTGAACTTA
CTATCAAAGATAAGAGACCATTTTTATCATTTCCTCTACTAATAATTTCCTTTGAATTT
TCTTCACACATACTTAGCAAAATTCCACAATTTTTACTTATAAATACACTACTTATCT
CACGTTTATAATCACAATAACTTAGATTTATTTTCTCTCCACTAAACCTAAAGAAAAA
TGGGGTTGTTCAACATCTCATTGTTACTCACTTGTCTCATGGTATTAGCCATATTTCAC
TCTTGTGAGGCCCAAAATTCACCCCAAGACTATCTTGCGGTTCATAACGATGCCCGT
GCCCAAGTCGGAGTCGGGCCTATGTCTTGGGATGCCAACTTGGCATCCCGAGCACAA
AACTATGCCAACTCAAGAGCTGGTGATTGTAACTTGATTCATTCTGGTGCTGGGGAG
AATCTTGCCAAGGGTGGTGGTGACTTCACGGGGAGGGCAGCCGTGCAATTGTGGGTG
TCCGAGAGGCCAAGCTATAACTACGCTACCAACCAATGTGTTGGTGGAAAAAAGTGT
AGACATTATACTCAAGTAGTCTGGCGCAACTCAGTCCGACTAGGTTGTGGTCGGGCA
CGTTGCAACAACGGATGGTGGTTCATTTCTTGCAACTATGATCCTGTAGGCAACTGG
ATCGGACAACGTCCTTACTAAAATGATGTATACTTATGACATGTTGCTAGTATTAAAT
AAAATTCTCATATGAGACGTCGAGAAGTTAAAATTTAAGTTTGACATATGAATCAAG
TCAAACTCCTATCTAAAATATTAAGGGATTAAATATTGAACATCTATAATTATTATTA
TTTCCCTTTTGATGTTGCTAATATGAATAATTCCACATACCATATGTTCATAATGGGC
TTAAGTTGATTATTAAGTACTGCATCTTCTTGTTTCCATAAAACATTAATATACATAA
AATTTTAATTAAGCATGGCATATATTAATTAGGCACATCAAGCACTTATCTAAACAC

GTAACTATTTATTCATAAATTCAGTTTCAACCATTCAAAATATTTGTTCTATTTTAAGT
GCCCATCAAAAAAAAATTCAATCATTACCCAATTTTTTTTCAATGACTAACAAATACT
CAAGTCTTCCATATTCTCTCATACAATAATGGCCTAAAACTATATTTTCATAAAATAT
TTTATAGGTGAAAATTGAATTTCATCCTTTCTTGACCTATTGTCTTAGATTTGAATACC
AGAAATAAAAACAAATATTGCTATTTATTGAAAAAATTTACGTAGCATGAAATTAAA
TAAATTGGCACATATTTAAAATATCTCGTCAGGAAAAAAAAATTGTACCCCTAACAA
ACGTTTAAATTTAATAATTTTCACAAATGTTACTAAATTTAACAATGAGAGTTTGATA
AATATCTAATGGTGAACAGAAAGTGTTCACTTTCGAAAAACAAAGAGGAGGAAAAG
TGATCTATTTTGACAAATTTAAGGATCAAAATTGATCCAATCATACATTAGGGACTAT
TTTGAATCTACTATAAAAATGATAAGGAACCATTTTTTTGTTTTTTCATTTTCTCTATT
AAGTTTCTTTGAATTTTCTTCACACATGCTAA

## Nitrogen Assimilation Genes

***GltS:*** Glutamate synthase – (aka. GOGAT) *Solanum lycopersicum* (Tomato)
- 78% similarity to Arabidopsis GLT1 (AT5G53460.1: NADH- dependent glutamate synthase). ammonia assimilation cycle, catalytic activity, nitrate assimilation, nitrogen compound metabolic process, nucleotide binding, oxidation-reduction process, oxidoreductase activity,.
- Role in plant: N response functions
- Primers designed with primer 3
  - F (5' -3'): CCACGACCTCCTTCTGAGAG
  - R (5' – 3'): TGGGCACACCATACATCATC
  - Product Length:160 bp

  - F (5' -3'): GTCCGCTCCTTACGACACAA
  - R (5' – 3'): CCAGTGCTTCACGCCATCTA
  - Product Length:144 bp
  -

DFCI – Tomato TC Report*: TC227398
GenBank accession number: DB678885


Nucleotide sequence:
AGGTTGAAGAGGAGCAGACATTGAAGAGGCCCATCCAAGTTGCTGAGGCAGTCAAG
CATCGAGGTTTTGTTGCTTATGAGCGACAGGGTGTGTCCTACAGGGATCCAAATGTT
CGGATGGAGGACTGGAAAGAGGTTATGGAGGAATCAAAACCCGGTCCGCTCCTTAC
GACACAATCTGCACGCTGCATGGACTGTGGAACTCCTTTTTGTCATCAGGAGAACTC
TGGATGTCCTCTTGGAAACAAAATACCAGAATTCAATGAGTTAGTGTATCACAATAG
ATGGCGTGAAGCACTGGATAGGCTTCTTGAGACAAACAACTTCCCTGAGTTCACTGG
TCGAGTGTGCCCTGCACCATGTGAAGGATCTTGTGTGCTTGGTATCATTGAGAATCCC
GTTTCTATCAAAAGCATTGAATGTGCCATTATTGACAAAGCTTTTGAGGAGGGGTGG
ATGGTGCCACGACCTCCTTCTGAGAGAACCGGGAGAAGAGTTGCAATTGTTGGAAGT
GGACCCTCAGGCCTGGCTGCTGCTGATCAGTTAAATAGATTGGGTCATACTGTCACC
GTGTTTGAACGTGCTGATAGGATTGGTGGTCTGATGATGTATGGTGTGCCCAACATG
AAGACCGACAAAATTGATGTCGTCCAGAGGCGGGTTGACCTTATGGA

*GS1***:** Glutamine synthetase – (aka. GLN) *Solanum lycopersicum* (Tomato)

- 78% similarity to Arabidopsis GSR1 (AT5G37600: glutamine synthetase). glutamate-ammonia ligase activity, seedling growth, aging , nitrate assimilation, ammonia assimilation cycle, high affinity for ammonium.
- Role in plant: N response functions.
- Potentially coexpressed with: TGA10
- Reference: Kumar et al. (2004)
- Primers designed with primer 3
    - F (5' -3'): GGACCTTCTGTTGGCATCTC
    - R (5' – 3'): GTGCTTCAAGCCAAGCTTCT
    - Product Length:225 bp

DFCI – Tomato TC Report*: TC218171
GenBank accession number: U14754

Nucleotide sequence:
CGCTGGAATTAACATCAGCGGGATCAATGGTGAAGTCATGCCGGGACAGTGGGAAT
TTCAAGTTGGACCTTCTGTTGGCATCTCAGCTGGTGATGAAGTGTGGGTAGCTCGTTA
CATTCTAGAGAGGATTGCAGAGATTGCTGGGGTGGTCGTGTCATTCGACCCCAAGCC
TATTCCGGGCGACTGGAATGGTGCAGGTGCTCACACAAATTACAGCACCAAGTCGAT
GAGGGAAGACGGAGGCTATGAAATAATCTTAAAGGCTATTGAGAAGCTTGGCTTGA
AGCACAAAGAACACATAGCTGCATATGGTGAAGGCAACGAGCGTCGTCTCTCTGGA
AAGCACGAAACAGCCAACATCAACACATTCAAATGGGGGGGTTGCAAACCGTGGTGC
ATCTGTCCGTGTTGGAAGAGACACAGAGAAGGCAGGCAAGGGATACTTTGAGGACA
GAAGGCCAGCCTCAAATATGGACCCATACGTCGTTACCTCCATGATTGCAGAAACCA
CCATCATCGGTTAACCTTGAAGACTTGATAGTATGAATTTGCTCGAGGGATCGCTTGT
TTCTGGTTTGCACAATTTGGGATAGGAGAAAAGATTGAATTGTGGAACGACCCTTTG
GACTTCACCTGTGTTATTTAGTTATAGGGATAGTTTGTCTCTGGTTATTTTTCTGTTTA
TTTGCCCCAGTTGAATTGTATTTTCATACAGCAAAGCCTTATTTCATTGCCTATGATTT
GGCAATGCTGTGTTACAAATGTTATTCTTATTAATAACAAAGATATTGAAAGGGTTTG
GTCC

**_NR:_** Nitrate Reductase - *Solanum lycopersicum* (Tomato)

- 67% similar to Arabidopsis NIA2 (>AT1G37130.1: Nitrate reductase 2, aka. NR2).
- Response to nitrate. Response to symbiotic fungus. Response to light stimulus
- Role in plant: nitrate reductase activity; nitrate assimilation
- Potentially coexpressed with: NiR; CIPK24
- Reference: Wang et al. (2001)
- Primer Reference: Balaji et al. (2008)
    - F (5' – 3'): CCCTCTGAGGATCAAGTCTTAGG
    - R (5' – 3'): CCCTACTTCATCGACTGTGCTAGT
    - Product length: 120 bp

DFCI – Tomato TC Report*: TC233150
GenBank accession number: X14060.1

Nucleotide sequence:
ATCCTGGTCTGACTGGACTGTGGAAGTTACAGGGCTGGTAAAACGACCAATGAAATT
CACAATGGATCAATTAGTTAACGAATTCCCTTCACGTGAATTGCCTGTCACACTTGTG
TGCGCAGGCAATCGTCGTAAAGAGCAGAATATGGTGAAGCAGACAATTGGTTTCAAT
TGGGGTGCTGCTGCCGTTTCAACCACCGTATGGCGCGGAGTACCTCTCCGCGCCCTGT
TGAAACGGTGCGGTGTTCAGAGTAAGAAAAAAGGCGCGCTTAATGTCTGTTTCGAAG
GTTCCGATGTTTTGCCTGGAGGTGGTGGTTCAAAGTACGGAACGAGTATAAAGAAGG
AATTCGCCATGGATCCATCTCGTGATATTATTGTAGCTTACATGCAAAACGGAGAAA
TGTTGTCACCGGATCATGGTTTTCCGGTAAGGATGATTATCCCCGGATTCATCGGTGG
AAGAATGGTGAAATGGTTAAAGAGGATTGTGGTCACTACACAAGAATCGGAAAGCT
ATTATCATTACAAGGACAATAGAGTCCTCCCTCCACACGTTGACGCGGAACTTGCCA
ACGCGGAAGCTTGGTGGTACAAACCAGAGTACATCATCAATGAGCTCAACATAAACT
CTGTCATTACAACTCCGTGCCATGAAGAAATTTTGCCCATCAATGCGTGGACTACTCA
GAGACCTTACACGTTGAGAGGCTATGCTTATTCTGGTGGAGGTAAAAAGGTAACTCG
AGTGGAAGTGACTTTGGATGGAGGAGAGACATGGAGTGTGTGTACACTTGATCACCC
AGAGAAGCCAACAAAGTATGGCAAGTACTGGTGTTGGTGCTTTTGGTCACTCGAGGT
TGAGGTGCTTGACTTGCTTAGTGCTAAAGAAATTGCTGTACGAGCTACCGATGAGAC
CCTCAACACTCAACCCGAGAAGCTTATTTGGAACGTCATGGGAATGATGAACAATTG
TTGGTTTCGAGTGAAGATGAATGTGTGCAAACCTCACAAGGGAGAGATTGGTATAGT
GTTTGAGCATCCGACTCAACCTGGAAATCAATCGGGTGGATGGATGGCAAAGGAGA
GACACTTGGAGATATCAGCAGTGGCTCCTCCAACACTAAAGAAGAGTATATCAACTC
CTTTCATGAACACAGCTTCGAAGATGTATTCCATGTCCGAGGTGAGGAAACACAACT
CTTCAGACTCTGCTTGGATCATAGTCCATGGACATATCTACGATGCCTCACGTTTCTT
GAAAGACCATCCCGGTGGTGTTGACAGCATTCTGATCAATGCTGGAACTGATTGTAC
TGAGGAATTTGATGCAATTCATTCTGATAAGGCTAAGAAGCTATTGGAGGACTTTAG
GATTGGTGAACTCATAACTACTGGTTACACGTCTGATTCGTCTCCAAACAGTTCTGTC
CATGGATCCTCTTCGATCAGTAGCTTCTTAGCACCTATTAAGGAGCTTGTTCAAACAC
CAACAAGGAGTGTAGCTCTCATCCCAAGGGAAAAAATCCCTTGCAAACTCGTCGACA
AGCAATCCATCTCCCATGATGTTAGGAAATTCAAATTTGCATTACCCTCTGAGGATCA
AGTCTTAGGGTTACCTGTTGGCAAACACATATTCCTCTGTGCCACAGTTGATGACAA
ACTCTGTATGCGTGCCTACACGCCTACTAGCACAGTCGATGAAGTAGGGTTCTTCGA
GTTGGTTGTCAAGATCTACTTCAAAGGTGTTCACCCTAAATTCCCTAATGGAGGTCAA
ATGTCACAACATCTTGATTCTCTCCCAATAGGTGCATTCCTTGACGTTAAAGGTCCAT
TAGGTCACATTGAATACCAAGGTAAGGGTAATTTCTTAGTCCATGGTAAACAAAAGT
TTGCCAAGAAGTTAGCTATGATAGCGGGTGGAACAGGTATAACTCCAGTATATCAAG

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TAATGCAATCAATATTGAAAGATCCTGAAGACGATACAGAGATGTATGTGGTGTATG
CAAACAGAACGGAGGATGATATTTTGCTCAAAGACGAACTTGATGCATGGGCAGAG
CAAGTTCCAAATAGGGTTAAAGTATGGTATGTCGTTCAAGAATCCATTACACAAGGA
TGGAAGTATAGTACAGGATTCGTTACAGAATCGATTCTTAGAGAACATATACCTGAA
CCATCTCATACAACATTGGCATTAGCATGTGGACCACCTCCAATGATACAATTTGCTA
TTAATCCAAACTTGGAGAAAATGGGATATGACATTAAGGAGGAACTATTGGTGTTCT
AAATTGGATGGTGATGATGATAGATGATATCTCTTTGGGAGGAAATAAATTCTTTT
GTATTTTCAGTTGTACTTTTTTTTTTTTTTTTTTTTTTGGTCATCCTTCTCTTCCTCGG
TTTCTTCCTCCGACTTCTCTTTGCTCTTGTCCTTCTTTTCTTTATCCTTTTTCTCTTTTTT
CTCCTTCTTGTCTATTACATTTTTTGTTTCATCCTCCGACTCCTCCTCACTCTCCTTGTT
CTTTTCTTTATCCTTCTTGTCTTTCTTTTGCTCCTTCATATCATCAGACTTTTCTATCTC
TTTCTCGACTTTGAGATGTTGTTCTTCCTTTTCCATCTTTTCTTCTTCTTTCCCGGGCAA
TAACAGTTGTTTCCGCTTCCTATCCAAAATTGTCCGCGTGTCAATGCGTCTTCAATTT
CTCCGAGATATCCTTTTCACTAAACACCAAGAAACTCAGT

***NiR***: Nitrite reductase – (aka Nii1) *Solanum lycopersicum* (Tomato)

- 72% similar to Arabidopsis NiR1 (At2g15620: Nitrite reductase)
- Role in plant: N response functions.
- Potentially coexpressed with: CIPK24, NR
- Reference: Kumar et al. (2004); Wang et al. (2001)
- Primers designed by Primer 3 Plus
  - F (5' -3'): AGCTCGTTCCCTGAAGATCA
  - R (5' – 3'): CGCCTTCCACAGTCTTCTTG
  - Product length 170

DFCI – Tomato TC Report*: TC232374
GenBank accession number: AW039265

Nucleotide sequence:
CAAATTTTCACCTGATCCACCTATTCTCATGAAAGGTTTAGTGGCTTGTACTGGTAAC
CAGTTTTGTGGACAAGCCATTATTGAAACGAAAGCTCGTTCCCTGAAGATCACCGAA
GAGGTTCAAAGGCAAGTATCTCTAACGAGGCCAGTAAGGATGCACTGGACAGGCTG
CCCAAATACGTGTGCACAAGTTCAAGTTGCAGACATTGGATTCATGGGATGCCTGAC
TAGAGATAAAGACAAGAAGACTGTGGAAGGCGCCGATGTTTTCTTAGGAGGCAGAA
TAGGGAGTGACTCACATTTGGGTGAAGTATACAAGAAGGCAGTTCCTTGTGATGAAT
TAGTACCACTTATTGTGGACTTACTTATTAAGAACTTTGGTGCAGTTCCACGAGAAAG
AGAAGAAACAGAAGATTAATAAAATTTGGATTAGATCATAATGATGGAATGTGCAA
TTATGTTTAGTGATTATGGAGGTATATAGCTAAGAGCTGGTTTGAATAATCAGAAAT
ATGTTGTGTTCATATCATTTATTGTACGATAAATCAACACAAACATTCCTACTTACCT
GAGAATATTACAAACTATATTCTTTGAAGC

*Housekeeping(Internal Control) Genes*

**Act:** Actin - *Solanum lycopersicum* (Tomato)
- Housekeeping Gene
- Role in plant: Structure in cytoskeleton.
- Primer References: Lovdal and Lillo, (2009); Dekkers et al., (2012); Yang et al., (2012)
  - F (5' – 3'): GAAATAGCATAAGATGGCAGACG
  - R (5' – 3'): ATACCCACCATCACACCAGTAT
  - Product length 159bp

  Actual primers used (modified from Lovdal and Lillo (2009)
  - F (5' – 3'): GGAATAGCATAAGATGGCAGACG
  - R (5' – 3'): ATACCCACCATCACACCAGTAT
  - Product length 159bp

DFCI – Tomato TC Report*: TC219951
GenBank accession number:  DB714861

 Nucleotide sequence:
TTGAGAGAAGGTTAGAATAGAGAAGAAGAAGAAGAAGAAAGAGAGCTTTTCCACAT
TTTGCATTCCCTGACTGTTTGCTAGTGTGTGCCCCCCCCTCTCTCTCTCCTCATCTCTC
TACCAATTTTCTCTCCGAAAAAAGGAATAGCATAAGATGGCAGACGGAGAGGATATT
CAGCCCCTTGTCTGTGACAATGGAACTGGAATGGTCAAGGCTGGGTTCGCAGGAGAT
GATGCTCCACGAGCTGTATTTCCTAGTATTGTTGGCCGCCCCCGCCATACTGGTGTGA
TGGTGGGTATGGGTCAAAAAGACGCCTATGTGGGAGATGAAGCTCAATCGAAGAGA
GGTATTTTAACTCTTAAATACCCAATTGAGCACGGAATTGTCAGCAATTGGGATGAT
ATGGAGAAGATATGGCATCATACTTTCTACAATGAGCTTCGTGTTGCCCCTGAGGAG
CATCCTGTCCTCCTAACTGAAGCCCCTCTTAACCCAAAGGCTAATCGTGAAAAGATG
ACCCAGATTATGTTTGAGACTTTCAATACCCCAGCTATGTATGTTGCTATTCAGGCTG
TACTCTCACTGTATGCCAGTGGTCGTACCACCGGTATTGTGTTGGACTCTGGTGATGG
TGTCAGCCACACTGTCCCAATTTATGAAGGGTATGCCCTTCCACATGCCATTCTCCGT
CTTGACTTGGCAGGACGTGACCTCACTGATAGTTTGATGAAGATCCTGACCGAGCGT
GGTTACTCGTTCACCACCTCAGCTGAGCGAGAAATTGTCAGGGACGTGAAAGAAAA
GCTCGCTTACATAGCTCTTGACTATGAACAGGAACTCGAGACTTCAAAGACCAGCTC
TTCTGTTGAGAAGAGCTATGAGCTCCCAGATGGGCAGGTGATCACCATTGGTGCTGA
GCGTTTCCGGTGTCCTGAGGTCCTTTTCCAACCTTCAATGATTGGAATGGAAGCTGCA
GGAATCCACGAGACTACATACAACTCTATCATGAAATGTGACGTGGATATTAGGAAA
GATCTTTATGGAAACATTGTGCTCAGTGGTGGTACTACCATGTTCCCAGGTATTGCTG
ATAGAATGAGCAAAGAAATTACTGCATTGGCTCCTAGCAGCATGAAGATTAAGGTG
GTCGCTCCACCAGAGAGGAAATACAGTGTCTGGATTGGAGGCTCTATCTTGGCTTCC
CTCAGCACCTTCCAGCAGATGTGGATTGCAAAGGCAGAGTATGACGAATCTGGTCCC
TCTATTGTCCACAGGAAGTGCTTCTAATTTTTCCAAGATTGACAATGTTGGTGAAAGG
AAAAGACTTCTTATTTCCTACTGGACCAGAGATGCAATTGTAGTGTTATATTCTGGCT
TTATTTTCTGTATTTTTGTTCTCATGTTGGATTGATGATATTGAGAGGGCAAAGGAGT
TAATTGTTGGGTTATGTTAATTCTTTTATTTCTAATGACTTTCTACTCTTTGTT

***Ubi*:** Ubiquitin - *Solanum lycopersicum (Tomato)*
- Housekeeping Gene
- Role in plant: Regulatory protein. Protein binding. Directs proteins to proteasome
- Primer References: Lovdal and Lillo, (2009); Dekkers et al., (2012)
  - F (5' – 3'): GGACGGACGTACTCTAGCTGAT
  - R (5' – 3'): AGCTTTCGACCTCAAGGGTA
  - Product Length: 134bp
- Primer Reference: Fiorilli et al. (2009)
  - F (5' – 3'): ACCAAGCCAAAGAAGATCAAGC
  - R (5' – 3'): GTGAGCCCACACTTACCACAGT
  - Product Length: 185 bp

DFCI – Tomato TC Report*: TC232697
GenBank accession number:  X58253

Nucleotide sequence:
GATATCTTTTTGTCATGATATAATATGTTTCTTTTAGCAAAAATGAAATAGTTCCGTC
ATTTATCGGATCATATTTAGATAGATTGTTGTAGATGACACTTTTATGAAATTACAAT
AATCATAAATTTATCACGGGCACAATTAGCGACAATGAAGACCAACCAAAGTTAGG
GGGCAAAAACCGAACAGGCCAACCAAAGTTATGGGTGTGCAAAAAATCGAATCGAT
CGATAAATCGAATCGAAAAAATGTTATTGGGTTATTTATGTTTTTTCGTGGGTTTATA
AAAAAAATTATTGAATTATTGGTTCGGTTTCAATTTTTATTATTGGGTTATTGGGTAA
ACCGATAATCCAATAAGACGGTAATAATTTTTATTTTACCCTTCATAATTATTTATTA
TTAGCAAGTTAATATATAATTAGACACTATAATTATATCAAATTATTAGTACTCTACC
AACTTCAGAGTTGGCTGATTTACTAGTTTTTATTGTTTATTCAAAACCTAAGAATTAA
AGTAATGCGTTCACAATTGCAGTTATTTGATTTTAATTTTAGTTTTAGTCTTATTGGAC
TGTTTTATTTTAGTTTTAGGTTATAATGGCAGGTTATAGCATTTGCATCTTAGTAAAG
GTCAGTAATTTGATTAACACAAAAAATTCCATACTATATTTTGGTGGTAATATGTAAT
TATAGCATTCGTGCTATTTTCTCGTATTGATACAATTTCTCATTATCTTTCTTGTTTTAC
TATATCAAAACATTTAGAGAAGTGAGAAGACATATAATATTTTACGGACATTTTCTT
ATTGGGTAAACTGAAAATCGAATCGATAATGATAAAAAACTGATAAATTGAAATCTG
ATAAAAAATATCTTGTTGATTTGTTATTGAATTAACATATTTAAAAGCTGAAAACCGA
TAAATCGAACGATAATATATAAAATCGAACCGAACCGATCGATGCACACCCTTGACC
AAATCTGAAGCACATATTTATCGATCTAAATTTTATTAAAGAGATTAATATCGAATA
ATCATATACATATTTCATATGTATAACAAATTTCAAATACACGTATCTAATATATCGA
GTGATGCGACAAATACATGTATCGGACGCACCAATTGATATAGAAAACGTAATATTG
AAAACTAATGTAAAGAAAAGTAACTTGATCCTAAACTAATCAAGATAAGCCCAATA
AATATACATTGTCATCTCCAAAGGCCCAAAAATGGCACAAGATGGCAGGCCCAATA
ACGAAGAAAAGGGCTTGTAAAACCCTAATAAAGTGGCACTGGCAGAGCTTACACTTT
CATTCCATCAACAAAAGAAACCCTAGAAGCCGCAGTGCCACTGATTTCTCTCCTCCA
GACGAAGATGCAGATCTTCGTGAAAACCCTAACGGGGAAGACGATCACCCTAGAGG
TTGAGTCTTCCGACACCATCGACAATGTGAAAGCCAAGATCCAGGACAAGGAAGGG
ATTCCCCCAGACCAGCAGCGTTTGATTTTCGCCGGAAAGCAGCTTGAGGATGGTCGT
ACTCTTGCCGACTACAACATCCAGAAGGAGTCCACTCTCCATCTCGTGCTCCGTCTCC
GTGGTGGTGCTAAGAAGAGGAAGAAGAAGACCTACACCAAGCCAAAGAAGATCAA
GCACAAGAAGAAGAAGGTTAAGCTCGCTGTGTTGCAGTTCTATAAGGTTGATGACAC
TGGAAAGGTTCAGAGGCTTCGTAAGGAGTGCCCTAATGCTGAGTGCGGTGCTGGAAC
TTTTATGGCTAACCATTTTGACCGTCACTACTGTGGTAAGTGTGGGCTCACCTACGTT
TACAACAAGGCTGGAGGCGATTGATTTTAATGTTTAGCAATGCTCTATCAGATTTTCT

TTTTGTCGAATGAACGGTAATTTAGAGTTTTTTTTTTGCTATATGGATTTTCGATTTTG
ATGTATGTGACAACCCTTGGGATTGTTGATTTATTTCAAAACTAAGAGTTTTTGCTTT
AATGTTCTCGTCTATTTTCGATATCAATCTTAGTTTTATCTCATTCTAGTTGTCTAATG
TTCAACATATTAGCAATTTGGCGGATTATAGAACTATCAAATATGCTTCTCAGGAAA
TTTGAGATTTACCAGTCCTTGTGCTCATGGGGTTGAGTATAATATAGGAAAAAATAG
TAAATTTAAGCCTGTGCTATGTTTCTATACTTTTATTTATTTGTCTCTGTACTTCCTCAT
GCTGAAACTCTGCTGTGCATTTCATTAATTTGAGAAACATAAATAAAGGGAACTGAG
AAGGGACTGCCTGTTTGGTTGTGTGTGCTACATTTAGTAATTCTGTAGTATAGATTGC
ATTATATGCTTTTAGCG

# APPENDIX A

## A.2: SUPLEMENTAL INFORMATION ON OTHER GENES CONSIDERED FOR USE IN CHAPTER 3

*Defense Response Genes*

*A-DOX1*:  Alpha-DOX1 - *Solanum lycopersicum* (Tomato) ,
- 71% similarity to Arabidopsis Alpha-DOX1 (At3g01420), response to oxidative stress, response to salicylic acid stimulus, response to other organism
- Role in plant: Defense response.
- Potentially coexpressed with: OSM, chiB, PR2
- Reference: http://atted.jp coexpression lists
- Primer Reference:  Primer 3 design
  - F (5'-3'): AAACCGGTCACTTGAACAGC
  - R (5'-3'): AGTCCAGCCCAAGTGTTACG
  - Product Length: 190bp

DFCI – Tomato TC Report*: TC221820
GenBank accession number: AY344539 (plus other ESTs)

Nucleotide sequence:
AATTGTCAAAGCCATCCAACATGCTAGTATTATTTATTCCCAATCGTGATAATATTAT
CTTAGTGCATTTCTTCTGCTTATGTTACTTTAAAAAATAAGTTATATATAGAGAGAGA
GGACGTTACCTTCCTGTACACTCCAAAAATCATATTACAAGTGATATTAATTTCTCGT
ATAAACAATGTCTTTTGTTATGCTCAAGAATCTCTTGCTATCCTCTCTCCGTAAATTC
ATCCACAAAGATTTCCATGAGATCTTTGACAAAATGACTCTCATCGATAAATTATTTT
TTTTGATTGTTCATTTTATTGATAAACATAACTTTTGGCACCGGCTACCGGTATTCTTC
GGGTTACTTTATCTTGGAGCACGGCGGAGTCTTCACCAGCAATATAATTTGATCAAC
GTCGGTAGAACACCTACCGGAGTTCGATCAAATCCGGCAGATTACCCTTACAGAACT
GCTGATGGAAAATTCAATGACCCTTTTAATGAAGGAACAGGCAGTCAATTTTCTTTCT
TTGGCAGGAATATGATGCCTCTTCATCAGAATAATAAGTTAAAAAAGCCAGATCCAA
TGGTAGTAGCAACGAAGCTTCTAGCACGAAGAAAATTCATAGACACTGGAAAACAA
TTCAATATGATAGCTGCTTCTTGGATACAATTTATGGTTCATGATTGGATCGATCATT
TGGAAGATACTCAACAGGTTGAGCTAAGGGCACCAAAAGAAGTTGCTAATGAATGC
CCACTCAAGTCCTTTAGGTTTAACAAATCCAAAGAAACTCCTACAGATTTTTATGAA
ATCAAAACCGGTCACTTGAACAGCCGTACTCCCTGGTGGGACGGAAGTGTAATTTAT
GGAAGTAACGAGGATGTTTTGAAGAAAGTGAGAACATTTAGAGACGGAAAACTGAA
ATTAGGTGAAAATGGACTCATCCAACAAGATGAAAATGGAAAAATTATCTCTGGTGA
TGTTCGTAACACTTGGGCTGGACTTTTAACGCTTCAAGCTCTCTTTGTTCAAGAGCAC
AATGCTGTTTGTGACACTTTGAAGAAAGAATATCCAGAATTAGAGGATGAAGAGTTG
TATCGTCATGCAAGGCTAGTCACTTCAGCTGTAATTGCAAAAGTTCACACCATAGAT
TGGACTGTTCAGCTTCTGAAAACCGATACTATGCTTGCAGGAATGCGTGCCAATTGG
TATGGATTACTAGGAAAGAAGTTCAAGGATACATTTGGTCATGTTGGTTCCATTTTAA
GTGGTGTTGTTGGAATGAAGAAACCTGAGAATCATGGAGTGCCTTATTCCTTAACTG
AAGAATTTACGAGTGTTTATAGAATGCATCAACTGTTACCTGATACACTTCAGCTAA
GAAATATAGATGCCACGCCTGGGCCAAACAAATCTCTTCCTTTAACTAATGAAATTC

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CCATGGAAGAAGTAGTTGGGAGTAAAGGAAAAGAGAATTTATCAAGAATTGGGTTT
ACTAAGCAAATGGTTTCAATGGGGCATCAAGCTAGTGGAGCTCTTGAGCTTTGGAAT
TATCCAGTGTGGATGAGAGATCTTATTGCCCAAGATGTTGATGGAACAGACAGGCCA
GATCCTATTGACCTTGCAGCTCTTGAAATTTATAGGGATAGAGAAAGAAGTGTTCCT
AGGTACAATGACTTTAGAAGAGGAATGCTTCAAATTCCTATTTCGAAATGGGAAGAT
TTGACAGATGATGAAGAAGCAATCAAAACACTTGGTGAAGTATATGATGATGATATA
CAAGAGTTGGATTTATTAGTGGGACTCATGGCGGAGAAAAAAATTAAAGGATTTGCC
ATTTCAGAAACAGCCTTCAACATATTCCTTCTCATGGCTATAAGGAGGTTAGAGGCA
GATAGATTTTTCACAAGCAATTACAACGATGAGACATACACAAAGAAAGGATTAGA
ATGGGTGAATACTACTGAGAGTTTAAAAGATGTGTTAGATCGTCATTATCCAGAAAT
GACTGATAAATGGATGAATTCAAACAGTGCCTTCTCTGTTTGGGATTCTTCTCCACAA
CCTCATAATCCTATTCCACTCTATTTTCGTGTTCCTCAGTAG

*CIPK24*: CBL-interacting protein kinase 24 - *Solanum lycopersicum* (Tomato)

- 70% similar to Arabidopsis CIPK3 (At2g26980: CBL-Interacting protein kinase 3). Response to abscisic acid stimulus, protein serine/threonine kinase activity, signal transduction.
- Role in plant: N response functions, response to hormonal substances.
- Potentially coexpressed with: NiR
- Reference: http://atted.jp coexpression lists

DFCI – Tomato TC Report*: TC223887
GenBank accession number: DB681252 (plus other ESTs)

Nucleotide sequence:
AAAATAAATATAATATAATAAATAGTAGTAGTATCATTTTTTCTTCAACCAAAGAAG
AAAATCACCATTAAAGACTTTTCCCATCAGTTTCGCCGCCCTTGTTGCCTTTCTTTCAT
CACAATCAAAATCCAATCTTTTTCTTCTTGTTTTCTTCCAAAGATCCAATATTTTCTCT
CCGATACACCCAGAAAAAGTGATATGTAACAGAATCCACCTTCCAATAATAGCAATT
TCATTTCTAAAAATACTTCTGATGGATTTTTTGTGTTTTTCGTTTCACTTCCCAAAAGG
GTTTTTGCTTTATCAAACATACCCTTTCCTATCAAACTTCTCATAATTTTTTTTTCTGA
ATTTTGGGATTTTTTAAAAACTCTTTTTTTTGTGTGTGTGTGGGGTGGAGAAAAGGTT
AAAGATGAGTATAGCCAAGTCCCAGGTTTGGCAACCTTGTAAAAAGAAGAGGATTT
AGCTTTAGGCATATAATTATATTAAGAAAATAAAAAGATTTCTGAAAAAAAGAAGA
AGAGGGATTTGAAGATCTATAAGGAAGAAGATTTGGATTGAATAAGGGAGATGGGT
TCAAGATCAAATAATGGAAGTGGGACTGGGAGGACAAGAGTGGGAAGGTATGAACT
TGGGAGGACATTGGGGGAGGGTACTTTTGCAAAAGTGAAATTTGCTAGGAATGTTGA
AACTGGTGATAATGTAGCCATAAAGATTCTTGATAAAGAGAAGGTCATGAAGCACA
AGATGATTGGTCAGATTAAACGGGAAATATCAACCATGAAACTTATTAGACACCCCA
ATGTAATCCGGATGTATGAGGTCATGGCCAGCAAGTCGAAGATATATATTGTTTTGG
AATTTGTTACTGGTGGCGAACTATTTGACAAAATTGCTAGTAAAGGTAGGCTCAAAG
AAGATGAAGCAAGAAAGTATTTTCAGCAGCTTATCAATGCAGTGGACTACTGTCATA
GTAGAGGTGTATTCCACAGAGACCTCAAGCCTGAGAACTTGTTATTGGATGCCAATG
GTGTTCTTAAAGTTTCGGATTTCGGATTGAGTGCGCTGCCTCAGCAAGTTCGCGAAG
ATGGACTTCTACATACAACATGTGGAACACCAAATTATGTGGCTCCAGAGGTGATCA
ACAATAAAGGTTATGATGGAGCTAAGGCTGATCTGTGGTCATGTGGTGTAATCCTTT
TTGTACTTATGGCTGGTTATCTACCTTTTGAAGAGTCAAATCTTGTGGCATTATATAA
GAAGATACATAAAGCTGAGTTTACATGTCCACCCTGGTTTTCCTCTAATGCAAAGAA
ACTGATCAAACGAATCTNNNNCCCNNTCCACAGACGCGCATCACANNACCNNNNCA
TTGANANGA

_**MKK2**_**:** Mitogen activated protein kinase kinase 2 - *Solanum lycopersicum* (Tomato)

- 65% similar to Arabidopsis ATMKK4 (>AT1G51660.1: mitogen-activated protein kinase kinase 4).
- defense response; triggers defense cascade
- Role in plant: MAP kinase kinase activity
- Potentially coexpressed with: MPK3; MPK1
- Reference: Pedley and Martin (2004); Xing et al. (2001)
- Primer reference: Li et al. 2012
  - F (5'-3'): TACTGATTTGACCCTTCCTCTTC
  - R (5'-3'): GGACGATGTAGAACCTTGTAAAC
  - Product Length: 186bp

DFCI – Tomato TC Report*: TC217324
GenBank accession number:  AY691331;  AI772376

Nucleotide sequence:
TATTTGTGTTTTGTGTTTTGTGTTGGATGGCTGCATTTCTCTTTCTCTCGATTTTTAGAA
CCTAAAAAAAAAATCCCCATTCATTCATTCGTTCGTTCATTCATTCTCTGTATAATTA
ATTTTGCAGACAATCCAATACCATTATTCAATCAATCATGCGACCAGCCGCCAACTC
CACCAACGCTGCATCATCCATGCCTCCTCCATCTTCCGCCGGGCAACGCAGTCGTCCC
CGCCGTCGTACTGATTTGACCCTTCCTCTTCCTCAACGTGACGTTGCTCTTGCTGTTCC
TCTCCCTCTTCCACCAACCTCTTCCTCATCCTCTTCCTCCCCGCTTCCTACCCCTTTAC
ATTTCTCTGAGCTCGAGAGGGTTAATCGCATCGGTAGTGGCACCGGGGGTACTGTTT
ACAAGGTTCTACATCGTCCCACTGGAAGACTCTATGCTTTGAAAGTGATCTATGGTA
ACCACGAGGATTCTGTCCGTCTTCAGATGTGCCGTGAGATCGAGATTCTACGAGATG
TAGACAACCCTAACGTCGTTAGGTGTCACGATATGTTCGATCACAACGGAGAAATCC
AAGTTCTTCTCGAGTTCATGGATAAAGGCTCTCTCGAAGGGATCCATATCCCTCTCGA
ACAACCTCTCTCCGATCTAACTCGACAGGTTCTATCCGGCCTCTATTACCTCCACAGG
CGTAAGATTGTTCACAGAGATATCAAACCTTCGAACCTCTTAATCAACTCCAGGCGT
GAGGTCAAGATTGCAGATTTTGGGGTCTCAAGAGTTCTGGCACAAACTATGGATCCT
TGCAATTCCTCAGTGGGTACCATCGCTTACATGAGTCCGGAGAGAATCAACACAGAT
CTGAATCACGGACAGTACGACGGGTATGCTGGGGACATATGGAGTCTTGGGGTGAG
CATCTTAGAGTTCTACTTGGGAAGGTTCCCCTTTTCTGTGGGGAGACAAGGAGACTG
GGCCAGCCTCATGTGTGCCATTTGTATGTCTCAGCCTCCTGAGGCACCACCCAGTGCT
TCTAGGGAGTTTAGGGAGTTCATTGCGTGCTGTTTGCAGAGGGATCCTGCCAGGCGG
TGGACGGCAGGCCAGCTATTGCGCCATCCCTTCATCACCCAGAATAGCACCGGCACC
ACCCACACGGGTCCTGCTACTACTACGACCTCACTGAGTCATCCATTGTTACCTCCAC
CTCCTCATTTTTCCTCCTCTTCTTGACGGTTTTAGGTTTGGAGAAATTCCCCACTCTCT
TTTGTTTTAGTTTTGTGGTTTTATTTTGGTGTAATGTTAAAATGTTGTTTACTGATGAT
GGATTTTTATTTGTGGGAGAAAGAAAAAATGGGGCTGGTTTTCCACTTTGAGGGAAG
TAGAAATATTATGGGTTGCCCCCAACTTAGTGATTCAATTAATGATATTTTGGATATT
TATTAAATCATCCTAAGTTTTGCTTGTGATTGATTGTTTGTGTATTGGATTTGTTCAAA
GTTACATTTAAATGGGGAAAAGAGATAAAAACTTTTGGGGGAGGAGCAAGTGTAG
TGTTGAAGATTAATCATTCTTTTATTATACTAGTTTAGGTGTTGGTTCCTCCTCCGTTA
AGAAGAAGGTAAAAAGGAAGGGAAAACTGTTGATCAATGAATTGTTTAAGTAACAT
GACTTGCGCTCATTCCTCTGCTATTCATAAAACAAGGATCACTCGACTTTTCCCTCTA
TCCCCTGTCCATCCTTTCCTTTCTTCATACATACATACATACAAGTTCTTTCCTTTGAA
AACTTTTCTATAAGTAAGTGATTATTCATATTTGTTGTGATAATGTTGCCTATTCAAA

AGTCTATAACAAGAGAGAGTATCACAATTCGCAAGTGTCGGCGAACTAGTCTCTCAT
GAAAAGTCGTTACAGTCACAATCACTTCCAATTTTTGTTTTCTTACTACATTATTATTA
TTATTATTATTG

*MPK1*: Mitogen-activated protein kinase 1 - *Solanum lycopersicum* (Tomato)
- 76% similar to Arabidopsis ATMPK6 (>AT2G43790 : mitogen-activated protein kinase 6).
- Response to multiple stresses including cold, osmotic stress, salt stress, oxidative stress, response to ethylene stimulus, response to jasmonic acid, involved in induced systemic resistance, defense response to bacteria
- Role in plant: MAP kinase activity; signal transduction
- Potentially coexpressed with: LeMKK2; MPK3
- Reference: http://atted.jp coexpression lists
- Primer reference: Li et al. 2012
  - F (5'-3'): CTGCGTCTTATTATGGAGTTGATTG
  - R (5'-3'): TAAAGAGGAAGTTGTCGGATATAGC
  - Product Length: 111bp

DFCI – Tomato TC Report*: TC217309
GenBank accession number:  AY261512

Nucleotide sequence:
TGAGGATTCATTTGTACTGTATTTTTCTGAACAAAATTATTTCCAAAATATGGATGGT
TCCGTTCCGCAAACGGATACGATGATGTCGGATGTGGCTGCACCTCCGGCTCAACAA
CCACCTCCGCCGTCACAACCGCTGGCTGGAATGGATAATATTCCGGCGACGTTAAGC
CATGGTGGCAGGTTCATTCAATACAATATTTTTGGTAATATTTTTGAAGTTACTGCTA
AGTATAAACCTCCTATAATGCCAATTGGTAAAGGTGCTTATGGAATCGTTTGTTCTGC
TTTGAATTCGGAGACAAATGAATCTGTAGCAATTAAGAAAATTGCTAATGCTTTTGA
TAACAAGATTGATGCTAAGAGGACTTTGAGAGAGATCAAGCTTCTTCGACATATGGA
TCATGAAAATATTGTTGCGATCAGAGATATAATTCCACCACCACAGAGAGAAGCCTT
TAACGATGTTTACATTGCGTATGAGCTTATGGATACTGATCTCCATCAAATTATTCGC
TCGAATCAGGGTTTATCTGAGGAGCACTGCCAGTATTTCTTGTATCAGATCCTCCGTG
GGTTGAAATACATACATTCTGCAAATGTTTTGCACAGAGACTTAAAGCCTAGCAATC
TTCTCTTGAATGCCAACTGTGATTTGAAGATATGTGATTTTGGGCTAGCTCGTGTCAC
TTCTGAAACTGACTTTATGACCGAATATGTTGTGACAAGATGGTATCGTCCACCTGA
GCTGTTGTTGAATTCATCCGACTATACTGCAGCAATTGATGTATGGTCAGTGGGTTGC
ATCTTCATGGAGTTGATGGACAGAAAACCCCTCTTCCCTGGCAGAGATCATGTACAC
CAGCTGCGTCTTATTATGGAGTTGATTGGCACTCCTTCAGAGGCTGAAATGGAATTTT
TAAATGAGAATGCAAAACGCTATATCCGACAACTTCCTCTTTACCGTCGACAATCAT
TTACTGAAAAGTTCCCGCATGTAAACCCAGCTGCTATTGATCTTGTCGAGAAAATGTT
GACATTTGATCCCAGAAGGAGAATAACAGTTGAAGACGCTCTTGCACATCCTTACCT
AACATCGCTCCATGATATCAGTGACGAGCCCATTTGCATGACTCCTTTTAGCTTCGAC
TTTGAGCAGCATGCGCTTACAGAGGAACAGATGAAGGAGCTAATTTACAGGGAGTC
GATTGCATTTAATCCTGAATACCAGCGCATGTGAATAATTGCTGACAGATTGTTGCA
GGTTTGATCTACATGTTATGTGTAACTGACAATATATCTCCCCATGTATATATGTGTG
CATTCCGTCCGGGAACATGGATGAGTTTCTTATGCAAACACTTAGTTATGAAGCTGA
CTTATGTATGGAGAGTTGTTTGATGTATCTTTATGGGGTGGATTTTATTTTAGACTGA
AATGAAAAATTCTGGTGGACAATATCTGTGTCGGATATTTCTGTTGTCATTGATATTC
TTATTTCATTGCCC

_**MPK3**_**:** Mitogen-activated protein kinase 3 - _Solanum lycopersicum_ (Tomato)
- 72% similar to Arabidopsis ATMPK3 (>AT3G45640.1: mitogen-activated protein kinase 3).
- Response to multiple stresses including cold, osmotic stress, salt stress, oxidative stress, response to ethylene stimulus, response to jasmonic acid, involved in induced systemic resistance, defense response to bacteria
- Role in plant: MAP kinase activity; signal transduction
- Potentially coexpressed with: LeMKK2; MPK1
- Reference: Pedley and Martin (2004)
- Primer reference: Li et al. 2012
    - F (5'-3'): TCTTCTTCTCATCTTCCTCCTTCC
    - R (5'-3'): CAGCACCCATATTAGCATCAACC
    - Product Length: 140bp

TCTTCTTCTCATCTTCCTCCTTCC
CAGCACCCATATTAGCATCAACC

DFCI – Tomato TC Report*: TC223280
GenBank accession number:  AW624703

Nucleotide sequence:
GTAACGCGTTCTCTTCTTCTTCTCATCTTCCTCCTTCCTTCCTGCAATTTTTCTCCAATC
AATCTCACATTATATATTCTCATAATTTTTTGATGAATTTATTAGTGTTTTACTAAATT
TCTATCAATAATGGTTGATGCTAATATGGGTGCTGCTCAATTTCCTGATTTTCCTAAA
ATTGTCACTCATGCTGGACAATATGTTCAGTATGACATTTTTGGTAATCTTTTTGAGA
TTACTAACAAGTATCAACCTCCTATCATGCCTATTGGACGTGGCGCTTATGGAATCGT
CTGCTCTGTGTTTAATGCGGAGCTGAATGAGATGGTTGCAGTTAAGAAAATCGCCAA
TGCTTTTGATAATTACATGGATGCTAAGAGGACGCTCCGTGAAATTAAGCTTCTTCGC
CATTTAGACCATGAAAACGTCATTGGTTTAAGAGATGTGATTCCTCCGCCCTTACGA
AGGGAGTTTTCTGATGTTTACATTGCTACTGAACTCATGGATACTGATCTTCACCAAA
TAATTAGATCAAACCAAGGTTTATCAGAGGATCATTGCCAGTACTTCATGTATCAGC
TTCTCCGTGGGCTAAAGTACATACATTCCGCGCATGTTATTCATAGAGATCTCAAACC
AAGTAACCTCTTGCTAAATGCAAATTGTGATCTTAAGATATGTGATTTTGGTCTTGCA
AGGCCAAACGTAGAGAACGAGAATATGACAGAATATGTAGTAACCAGATGGTACAG
AGCACCGGAGCTTTTGTTGAACTCTTCAGATTACACTGCTGCCATAGATGTTTGGTCT
GTGGGTTGCATCTTCATGGAGCTTATGAATAGAAACCTTTGTTTGCTGGAAAAGAT
CATGTACATCAAATTCGCTTGCTAACTGAGCTTCTTGGCACTCCTACAGAATCTGATC
TTAGCTTCCTCCGTAATGAAGATGCAAAAAGATACGTCAGGCAACTCCCACAACATC
CACGCCAGCAGTTAGCAACAGTGTTCCCTCATGTGAATCCATTAGCCATTGATCTTGT
AGATAAGATGTTGACGCTCGACCCTACTAGAAGAATAACAGTTGAGGAAGCATTAG
CTCATCCCTACCTCGCAAAGCTCCATGATGCAGCTGATGAACCAGTCTGCCCCATCCC
GTTCTCTTTCGACTTTGAGCAACAAGGGATAGGAGAAGAGCAGATTAAAGACATGAT
TTATCAAGAAGCTTTGGCGTTGAATCCTGAATATGCTTAAGCATAAGAGAAATCAGT
TCTTCTCTTGCTTACCTAGTGTGGATTTTCTTGCTTGGACCAGCCCTCTCAAAGTTTTT
GTTCACAGGGTCAGTAGTAGTCCTTTGCAACTTTAATGTAAGGCAGCCTTCAATTTGC
AGCCATTTTTATATACCTTTCTTCTTTATTTTTTTTACTTAATTATGGTGGTGTTCGGGC
TAGCTTGTGTTCACTCGACTATTCCATAGGATACATGTCATCTCCCACCAACAAAACG
TTTGT

*PAL*: phenylalanine ammonia lyase- *Solanum lycopersicum* (Tomato)

- Primers designed using primer 3 plus
  - F (5' -3'): GCGTGGCTGGTATTAGTGGT
  - R (5' – 3'): GGCTTTCCGTTCATCACTTC
  - Product length 178

DFCI – Tomato TC Report*: TC224223
GenBank accession number:  M83314.1

 Nucleotide sequence:
CTGCAGGTCAACGGATCATATTCTACACATATATAATGCACTCCAATTGACATAATA
CATAACGTGACATATGATACATTTATTAATATTAATTGTCACATTTACACTTCACATA
TTAAAATACTCTCGTATGAATGCAATTTGAAACATATTTTAAATTAATTGATTGATAT
ATATTGAACAAAACCTAACAAAAATGCACCCTCTTGGTTCACAAAGAAACTTTCTTC
TATTTCTCACTTATTTCTGCTAGTGTCTTTCCTATTCAAAGCCATCATTTCCATCAACC
TTCACAATACCATGTTTAAAAAGTCATTAAAAATCAATTTTTTAAATAGAAAAAAAC
AAGAAGATGGAAATCACTTGGTTGGTACTATATATTTAGTTGTTAAGTTTGACTCATA
CCGTGTATTGACCAATATAAATAAAATCTTATTTCAAATAAATTCAAAAGTTCAATA
AATATATATTCGTTCATAACTTATAATAAAATTGATTATACATAGTCCTCCCCCATTC
ACTTTTACTGATCAATTATTTCTAAAATATATTATTACTTTTACTTGTTATTTTTAATA
AATTAAGAAAATATAATACTCCCTTCGTTTTTAAAAAAATACCTAGTTTGACTTGAAA
CGGAGTTTAATAAAAGAAAGAAGACTTGTTAATCTTGTGATTCTAAATTAAAGTTAT
GTCAAATGTACCAAAATGTCCTTTAATCTTGTGGTCTTAAACATGTCACATGAAAAAT
TAAAGTGTTTCCAAAAAAAGAAAGGGGTCAATGTCATTCTTTTTTAAACAGACTAAA
AAAGAAATAAACTCATTCTTTTTGAAACGGAGAGAGTAATTTTTTCCACGTTTTACTC
ATTAATATTAAATATTATTCTCTAGATCATCCTATAAGATCTAATAGTGGACATCAAT
TAATACCTATGTCACTTATTATTATTTTAATAATTGTATCAAGTCAAATAATAACAAG
TAAAAATGGAGTACCTACTATTAATCTTCAACAACCACAATTTACTAGTTTTTTCCTA
GCAACCCCCTCTCACATATTTCACCATTTACTGGTTTTTTCCTAGCAACCCCCTCTCAC
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CATATATATACCACAACCAAAACAAAAGGTTTTATAAGTTCACAACATTTTTTATATA
CATACAAATAAACTCTAACCATTTTCTCTTCACTAAAATTTCTTCATTACAAATCTAA
CAATTTACTTGATCCAATGGCACCATCAATTGCACAAAATGGACATATTAATGGAGA
AGTAGCTATGGATTTGTGCAAGAAATCAATCAATGATCCATTGAATTGGGAAATGGC
TGCTGATTCTTTAAGAGGCAGCCATTTGGATGAAGTGAAAAAGATGGTGGATGAATT
TAGAAAGCCAATTGTGAAACTTGGGGGTGAAACTTTGTCAGTTGCACAAGTTGCATC
CATTGCAAATGTTGATGACAAAGTAATGGGGTTAAAGTGGAACTTTCTGAAAGTGC
AAGGGCTGGTGTGAAAGCTAGTAGTGATTGGGTTATGGATAGTATGAGTAAAGGTAC
AGATAGTTATGGTGTTACTGCTGGATTTGGAGCAACATCTCATAGAAGAACAAAAAA
TGGTGGTGCTCTTCAAAAAGAACTTATTAGGTAAACAAACTATTTTTTTTCGTTATAT
ATACTAACAATGTAAAGAATTTAATATTTTTTTGTTATATATACTAACAATGTAAAAA
ATTTAATATTTTTTTGTTATATATACTAACAATGTAAAGAATTTAATATTTTTTTGTTA
TACATAGCTTATCGACTACTTAAGTGCTCCATTGATAAAGATTTTTTTTTGTTTTTACG
CGAAGGGGATTCGGATGAATTCAGTTAAAATGTGATCTTAATGAATTATGATATTTTT
TTGTAGGTTCTTGAATGCTGGAGTTTTTGGTAATGGAATAGAATCATTTCACACATTG
CCACATTCAGCAACAAGGGCAGCTATGCTTGTTAGGATCAACACTCTGCTTCAAGGC
TACTCTGGCATTAGATTTGAGATCTTGGAAGCAATCACTAAGTTGATCAATAGCAAC
ATCACCCCGTGTTTGCCTCTCCGTGGCACGATCACTGCCTCGGGTGATCTCGTCCCTT

TGTCCTATATTGCTGGTTTGCTCACTGGCAGACCTAATTCCAAGGCTGTTGGACCCAA
TGGTGAGAAACTTAATGCTGAGGAAGCTTTCTGCGTGGCTGGTATTAGTGGTGGATT
TTTCGAGTTGCAGCCTAAGGAAGGACTTGCACTTGTGAATGGCACAGCAGTTGGTTC
TGCTATGGCATCAATAGTCCTGTTTGAGTCCAATATCTTTGCTGTTATGTCTGAAGTT
TTATCAGCGATTTTTACTGAAGTGATGAACGGAAAGCCCGAATTCACTGACTATTTG
ACACACAAGTTGAAGCATCACCCTGGTCAGATTGAGGCTGCTGCTATTATGGAACAC
ATTTTGGATGGAAGCTCTTATGTGAAGGTAGCTCAGAAGCTCCATGAAATGGATCCT
CTTCAAAAACCAAAGCAAGATCGTTATGCTCTCCGAACATCTCCACAATGGCTTGGA
CCTCAGATTGAAGTCATTCGTGCTGCAACTAAGATGATCGAGAGGGAGATTAACTCA
GTGAACGACAATCCATTGATCGATGTTTCAAGAAACAAGGCCTTACATGGTGGCAAC
TTCCAAGGAACCCCTATTGGTGTCTCCATGGATAATACAAGATTGGCCCTTGCATCA
ATTGGTAAATTGATGTTTGCCCAATTCTCAGAGCTTGTCAACGACTATTACAACAACG
GGTTGCCATCTAATCTGACAGCAGGAAGGAATCCAAGCTTGGACTATGGTTTCAAGG
GCGCTGAAATCGCGATGGCTTCTTACTGCTCGGAACTTCAATTCTTGGCAAATCCAGT
GACTAACCATGTCTAAAGTGCTGAGCAACACAACCAAGATGTGAATTCCTTGGGCTT
AATTTCAGCCAGGAAAACAGCTAAGGCTGTTGATATCTTGAAGATAATGTCATCAAC
CTATCTCGTGGCTCTTTGCCAAGCTATTGACTTACGACATTTGGAGGAAAACTTGAAG
AGTGTTGTCAAGAACACAGTTAGCCAAGTAGCTAAGAGAACTTTGACAATGGGTGCT
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AGAAGCTGAGACAAGTCCTTGTTGATCAAGCAATGAAGAATGGTGAAAGTGAGAAG
AATGTCAACAGCTCAATCTTCCAAAAGATTGGAGCTTTCGAGGACGAATTAATCGCT
GTGTTGCCTAAAGAAGTTGAGAGTGTAAGAGCTGTTTTTGAAAGTGGCAACCCTTTA
ATTCGTAACAGGATCACAGAATGCAGATCATATCCATTGTACAGGTTGGTGAGAGAA
GAACTTGGAACAGAATTGTTGACGGGTGAAAAAGTTCGATCACCTGGTGAGGAGATT
GATAAAGTGTTTACAGCAATATGTAATGGACAGATTATTGATCCATTGTTGGAGTGT
CTGAAGAGCTGGAATGGTGCTCCTCTTCCAATCTGCTAAATGTGTTATTCTTTCAAGT
TCTTTTTTTGTACCTTTTAGTGAATTACTAGAATTATAATGATGTTATGAACTTATATT
AAAAAAAAATATTTTTGACTATAAAATTTAGTTTTGTTATTGAAATTAAAGGCTCAAT
CTGTGTTCTTTCCTTCTGTTATCTGAATATTATAAGAATTCAAGTAATCTTTTAGCTTT
GTGAACATGATGACATGCTTTCTT

**_PAL5_**: phenylalanine ammonia lyase 5- _Solanum lycopersicum_ (Tomato)

- Primers from Lovdal et al. 2010
  - F (5' -3'): TTTCTCCATTACAAATCAAACCA
  - R (5' – 3'): TTCACTTCATCCAAATGACTCC
  - Product length 178

DFCI – Tomato TC Report*: TC233801
GenBank accession number:  M90692.1

 Nucleotide sequence:
TAATTAATCTTCCAACAACCACCATTTTTAGTCATTTCCTACAACCCCCTCTCACATA
ATTTTCTTTACCTACCATCCTTTGTTCCTCTCTATATATACTCACCACATATATCATCT
ACCATAACCAAAAAAAATAATAATAATACACTAATCATAGTTCACAACATATTTTTT
TTTATATATATAAATAAAATTCCCATTTTTTCTCTTCTCCAAATTCTCCTAAGTAAAAT
TTCTCCATTACAAATCAAACCATTTTTTGTTGGTCCAATGGCATCATCAATCGTACAA
AATGGACATGTTAATGGAGAAGCTATGGATTTATGTAAGAAATCAATTAATGTTAAT
GATCCATTGAATTGGGAAATGGCTGCTGAATCATTAAGAGGGAGTCATTTGGATGAA
GTGAAAAAGATGGTGGATGAATTTAGAAAGCCAATTGTGAAACTTGGGGGTGAAAC
TTTAACAGTTGCACAAGTTGCATCCATTGCAAATGTTGATAACAAAAGTAATGGGGT
CAAAGTGGAACTTTCTGAAAGTGCAAGGGCTGGTGTGAAAGCTAGTAGTGATTGGGT
AATGGATAGTATGGGGAAAGGTACAGATAGTTACGGTGTGACTACTGGATTTGGTGC
AACATCTCATAGAAGAACTAAAAATGGCGGTGCACTTCAAAAGGAACTTATCAGGT
AAATTTTTTTTTTTATGTATATACACTCGATAACGTAAAGAATTTTTATATTTTTTTCG
TATTTATAGTTTGTTATGTCAATTATGATATATATCTATAGTTTTCTTCATGTGATCTA
ATAGTAGTGTTAAGTTTACTACAATTATTTGATTTGATAGCGTAAAAAATTCTTTTTT
AATAAATAGCAATTTATTTATTTAATTTCGAATTATCATAGTAAAATTTGATTATTGA
AAGCTATGTATTTAATTATACAAGTTGAACCACCCTATTGATTTGTGCATGTGGGGTT
TGGGAAAAGTTAGTGTGACTGTGTGGTAGGTGAGGGAGATTTTGTCATTTTACTTCCT
TCAATTTTTGTTTTTTTATCAACTTGGACGTTAGTAAGTAATTTTTTTCAAATTGGTTT
TCTTCATTTATATGTGAATAAATAATTCTAGTAAGAAATCTTTATTTTGAATAAATAA
AACTATAGTATCTTGTGTTTTCATCACAAAAATATATGAGACAGATTGTTCAACTTTT
TAAGATGTAATGTTAGTTCCCATGTGCCATCTCTTGTCAGATATGTATTTTATAGTGT
CACATATAAAAATATTATAAATCAGTATTTTTTTTTTTATAATCAAATTTGTGCTCATG
ATCTTTATTTGTAGAATTTTAGTTAAAAATGTGATGTTGATGAATATTATGATTTATTT
TCTTCAGGTTCTTGAATGCTGGAGTTTTTGGAAATGGAACAGAATCATCTCATACTTT
GCCACATTCAGCAACAAGGGCAGCTATGCTTGTTAGGATCAATACTCTGCTTCAAGG
CTACTCTGGCATTAGATTTGAAATTTTGGAAGCAATTACTAAGTTGATCAACAGCAA
CATTACCCCGTGTTTGCCCCTTCGTGGGACGATCACTGCATCGGGTGATCTTGTCCCG
TTGTCATACATTGCTGGTTTGCTCACTGGCAGGCCTAACTCCAAGGCTGTTGGACCTA
ATGGTGAGAAACTTAATGCTGAGGAACGGTTCCGCGTGGCTGGTGTTACTAGTGGAT
TTTTCGAGTTGCAGCCCAAGGAAGGACTTGCACTTGTGAATGGTACTGCAGTTGGTT
CTGGTATGGCATCAATGGTACTGTTTGAGTCCAACATTCTTGCTGTCATGTCTGAAGT
CTTGTCAGCGATTTTCGCTGAAGTGATGAACGGAAAGCCTGAATTCACTGACTATTT
GACACACAAGTTGAAGCATCACCCTGGCCAGATTGAGGCTGCTGCTATTATGGAACA
CATTTTGGATGGAAGCTCTTATGTCAAGGCAGCTCAGAAGCTCCATGAAATGGATCC
TCTTCAAAAACCAAAGCAAGATCGTTATGCTCTTCGAACATCTCCACAGTGGCTTGG
ACCTCAAATTGAAGTCATCCGTGCAGCAACAAAGATGATTGAGAGAGAGATTAACTC
AGTGAACGACAACCCATTGATCGATGTTTCAAGAAACAAGGCCTTACACGGTGGAA

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ACTTCCAAGGTACCCCTATTGGTGTGTCCATGGATAATACAAGATTGGCCCTTGCATC
TATTGGGAAATTGATGTTTGCCCAATTTTCGGAACTTGTCAACGACTATTACAACAAT
GGGTTGCCACTCAATCTCACAGCTGGAAGGAATCCAAGCTTGGATTATGGACTCAAG
GGTGCTGAAATTGCAATGGCTTCTTACTGCTCGGAACTTCAATTCTTGGCAAATCCAG
TGACTAACCATGTTCAAAGTGCTGAGCAACACAACCAAGATGTGAATTCCTTGGGCT
TAATCTCAGCAAGGAAAACAGCTGAGGCTGTTGACATCTTGAAGCTAATGTCATCAA
CCTATCTCGTGGCGCTTTGCCAAGCTATCGACTTGAGGCATTTGGAGGAGAATTTGA
AGAATGCTGTGAAAAACACTGTAAGCCAAGTAGCCAAAAAGACTCTGGCAATGGGT
GCTAATGGCGAACTTCATCCAGCAAGATTCTGCGAAAAGGAATTGCTACAAGTGGTG
GAAAGGGAATATTTGTTTACCTATGCTGATGACCCCTGCAGCTCCACCTACCCTTTGA
TGCAGAAGCTGAGACAAGTCCTTGTTGATCATGCAATGAAGAATGGTGAAAGTGAG
AAGAACTTGAACAGCTCAATCTTCCAAAAGATTGTAGCTTTCGAGGACGAATTGAAG
GCCGTGTTGCCTAAAGAAGTTGAGAGTGCAAGAGCTGTTGTTGAAAGTGGAAACCCT
GCAATTCCTAACAGGATCACAGAATGTAGATCATATCCATTGTACAGGTTGGTTAGA
CAAGAAGTTGGAACAGAACTATTGACAGGTGAAAAAGTTCGATCGCCCGGTGAGGA
GATTGATAAGGTATTCACAGCATTTTGCAATGGACAAATCATTGATCCATTGTTGGA
GTGTCTCAAAAGCTGGAATGGTGCTCCTATTCCAATCTGCTAAATGTTTGTCAACTGT
TACTTTCAAATTCTTTTTTTTTACCTTACAGTAATTTACTATAATCATAATATCATTTTA
TTGTATTTTGGCCATATGTAATATTGTCTATCAATATAAGATTTTTGCTATTAGATTGT
TTAATCTTAAAACAACATATTAGATGATTATCATTTAACATTAACAATAACGTACCCA
ATTTTATATTAAGATTTAACAATTTTTAATCACGCCCACTACGACAAAAACAGCTTTT
AGCGATATTAAATATTGACAT

**PR1a:** Pathogenesis-related leaf protein 4 precursor (aka *PR-4*) - *Solanum lycopersicum* (Tomato)

- 60% similar to Arabidopsis ATPR1 (>AT4G33720.1: CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein) superfamily protein).
- Role in plant: Pathogenesis related protein.
- Potentially coexpressed with: LeMKK2; MPK3; MPK1
- Reference: Block et al. (2005); Lochman and Mikes (2006)
- Primer Reference: Aime et al. 2008
  - F (5' -3'): TCTTGTGAGGCCCAAAATTC
  - R (5' – 3'): ATAGTCTGGCCTCTCGGACA

DFCI – Tomato TC Report*: TC221944
GenBank accession number: AW034882

Nucleotide sequence:
GAAAATGGGGTTGTTCAACATCTCATTGTTACTCACTTGTCTCATGGTATTAGCCATA
TTTCACTCTTGTGAGGCCCAAAATTCACCCCAAGACTATCTTGCGGTTCACAACGATG
CCCGTGCCCAAGTCGGAGTCGGGCCAATGTCTTGGGATGCCAACTTGGCATCCCGAG
CACAAAACTATGCCAACTCAAGAGCGGGTGATTGTAATTTGATTCATTCTGGTGCTG
GGGAGAACCTTGCCAAGGGTGGTGGTGACTTCACGGGGAGGGCAGCCGTGCAATTG
TGGGTGTCCGAGAGGCCAGACTATAACTACGCTACCAACCAATGTGTTGGTGGAAAA
ATGTGTGGACATTATACTCAAGTAGTCTGGCGCAACTCAGTCCGACTAGGTTGTGGT
CGGGCTCGTTGCAACAATGGGTGGTGGTTCATTTCTTGCAACTACGATCCTGTAGGC
AACTGGGTTGGAGAACGTCCTTATTAAAGTATCGTCTATTTCTGACATGTTGCTAGTA
CTAAATAAAATTTCCATATTACATGTCTAGGAATTAAAATGATAAGTGGATCGGATT
GATATCCTATTATTATTGTTGTTGTTGTTGTTTCCCTTTGATGTTGCTAGTATGAATAA
TTCCACGTACCATATGTTCATGGTATCGTGGCTTAGGTTCTTTACTTTTTCAAAATATG
AAATTTATATATTTA

*PR2*: Pathogenesis-related protein P2 precursor - *Solanum lycopersicum* (Tomato)
- 73% similarity to Arabidopsis PR4 (At3g04720: Pathogenesis Related Gene 4), chitin binding, systemic acquired resistance, response to ethylene stimulus, defense response, response to virus.
- Role in plant: Defense response.
- Potentially coexpressed with: A-DOX1, OSM, chiB
- Reference: http://atted.jp coexpression lists
- Primer reference: Balaji et al. 2008
  - F (5'-3'): TTTACTGCGCTACCTGGGAT
  - R (5'-3'): ATCTACCGCATGAAGCTTGG
  - Product Length: 111bp

DFCI – Tomato TC Report*: TC223627
GenBank accession number:  BT013355

Nucleotide sequence:
GAAATTAAAAAATTACAATATTTATGGAGAGAGTTAACAAGTTGTGTGTAGCATTTT
TTGTCATCAACATGATGATGGCGGTGGCCGCAGCGCAAAGCGCTACGAACGTTAGGG
CAACGTATCATTTGTACAATCCGCAAAACATAAACTGGGATTTAAGAACTGCTAGCG
TTTACTGCGCTACCTGGGATGCTGACAAGCCTCTGGAGTGGCGCCGGAGGTATGGCT
GGACCGCTTTTTGCGGTCCAGCTGGACCTACGGGCCAAGCTTCATGCGGTAGATGCT
TGAGGGTGACCAACACAGGAACAGGAACACAAGAAACAGTGAGAATAGTAGATCA
ATGCAGAAATGGAGGGCTTGATTTGGATGTAAACGTTTTCAACCGATTGGACACTAA
TGGATTGGGCTATCAGAGGGGAAACCTTAATGTTAACTATGAATTTGTCAACTGCTA
AACTTAAAAAAAGTGTCATATATCATCATTACTATAATAAAATAATAAATCACGATC
TAAATTGATTTCATAGTACGTACTATCTTAAAGTTAGTAAAAGAAATCGAGCTAACT
TTTAATACTACTCATATATAAAAGTTCTACATGTATTTTGTATATCCTTCACGATTAAT
GAAATAAATCTTATTATTATTATCATG

***TGA10*:** TGA10 transcription factor - *Solanum lycopersicum* (Tomato)
- 57% similarity to Arabidopsis TGA3 (At1g22070: TGA1a-related gene 3). DNA binding, transcription factor activity, systemic acquired resistance, salicylic acid mediated signaling pathway, possible response to bacteria.
- Role in plant: defense related.
- Potentially coexpressed with: GLN
- Reference: http://atted.jp coexpression lists

DFCI – Tomato TC Report*: TC236528
GenBank accession number(s): BW685052, EG364339

Nucleotide sequence:
ATCGGCCGAATTGAGTTTGCTTCTATCATCAATTATCAAAAAAGAAAATAATGGGTC
TTCAAAGTCATGAAAATCAAATAACTTTTGGAAATCATGATCATCAGTTTCATCAAC
AACAACAACAAACTCAACATCAGCATCAACACCAACAACTATTATTTCAAAATAATT
CTGATCAGCGAAGTAACGCTGATCAGATTTCTTTTGGAATGTTACATCAGTCATCTTC
TGTCATACCTGAAAATTTCATAAATAAAGAGAGTAGCAGTACTGGAGGTTATGATTT
AGGTGAACTAGATGATCAAGCACTTTTCCTTTACCTTGATGCTCAAGATCCTTCTTCT
AATCATGATCAAATACAAAATAATTCAGAGATGATGAGACCACCAACTCTCAACATT
TTTCCATCACAACCCATGCATGTTGAGCCATCATCCACAAAGGGAAATACTGGATTT
GTTTCTAGTGGTTCTGAAAAATCATCTGAGCCATCCATAATGGAGTTATCAAAATCC
AAAAATAATGTACTTTCTACTTCTTCTGGACCTGAACCTAAAATTCCTAAGCGAGAGT
GGAATAGGAAAGGTCAAAATTCAGGTTCAGAGCAAAATGCACCTAAAACAACAGAT
CATAAGACATTGAGGAGACTTGCTCAGAATAGAGAGGCAGCTAGGAAAAGCAGAAT
TAGGAAAAAGGCTTATATTCAACAACTAGAGTCAAGTAGGATAAGGCTTGCCCAGCT
AGAGCAAGAACTACAAAGAGCTAGATCTCAAGGTTTTCATTTTGCGGGTACTCTTTT
AGGAGGAGACCAAGGACTTCCTAATAACATTGCAAACATGACCTCAGAAGCTGCAG
CGTTTGATATGGAGTACTCAAGGTGGCTAGAAGAACATCATCGTCTCATGTGTGAGC
TTCGAAATGCAGTGACTGAACATTTTCCGGAGAACGATCTTCGAATTTACGTCGAAA
ATTGTGTCACACATTACGATGAAATGATGAATCTCAAGAGCATACTTTTAAAAACCG
ATGTCTTTCATCTTATTTCCGGCATGTGGAGAACTCCGGCGGAGCGTTGTTTCATTTG
GATGGGAGATTTCCGCCCATCCGAGCTTATCAAGATAATCTTGAGTCAAATAGAGCC
ATTAACAGAACAACAA

_Nitrogen Assimilation Genes_

**_NRT1.2:_** Nitrate Transporter 1.2 - _Solanum lycopersicum_ (Tomato)
- 71% similar to Arabidopsis NRT1.1 (AT1G12110.1:NRT1.1 Nitrate transporter 1 gene). Response to jasmonic acid stimulus, response to nitrate.
- Role in plant: nitrate transmembrane transporter activity
- Potentially coexpressed with: --
- Reference: Wang et al. (2001)
- Primers Designed in Primer 3
  - F (5' -3'): CTCCCTGATGCTTGGGATTA
  - R (5' – 3'): CCAAGCAAAGTGAGCATGAA
  - Product length: 230 bp
-

DFCI – Tomato TC Report*: TC241078
GenBank accession number(s): X92852, AW979368; AW219289;

Nucleotide sequence:
TCATCAATCTTCTTTTAGCTCTCAACAAAAATAAATAAAAATACACCTTTCATTTTTT
TTTTTAATAATTCTCATTGCTCATTGGATCCAATTTTGTGGCTAAAAAGTAAATATTA
GAATATTTGCAAAAATGTCACTTCCTGAGACACAAAATGATGCAAAAACTCTCCCTG
ATGCTTGGGATTACAAAGGAAGGCCATCTCTTAGATCCTCCTCTGGTGGTTGGGCAA
GTGGTGCAATGATTTTAGGTGTTGAAGCTGTGGAGAGGCTAACCACACTAGGTATTG
CTGTAAACTTGGTGACTTATTTGACTGGAACTATGCATTTAGGCAATGCTACTGCAGC
CAATAATGTCACCAATTTTCTTGGAACTTCTTTCATGCTCACTTTGCTTGGTGGTTTTA
TTGCTGACACTTTCCTTGGAAGGTATCTTACAATTGGAATATTTGCCACAGTTCAAGC
AATAGGTGTTACAATCTTGACCATTTCGACCATAGTCCCAAGTCTGCGACCACCAAA
ATGTGAACAAGGTAGCTCATCATGCATCCCGGCTAACGGCAAACAACTCATGGTCCT
ATACATCGCGCTATACATGACCGCCCTTGGTACGGGCGGTCTAAAGTCGAGTGTATC
GGGATTCGGGACCGACCAATTCGACGATTCGATCGAAAAGGAAAAAGGACAAATGA
TAAAATTCCTTGATTGGTTCTTTTTCTTCATAAATGTTGGTTCACTAGGTGCAGTCAC
AATATTGGTCTATGTACAAGATAATTTGGGAAGAGAATGGGGTTATGGAATTTGTGC
ATGTGCAATTGTTATTGCACTTGTATTATTCTTATTTGGTACAAGAAAGTATAGGTTT
AAAAAACTTGTTGGAAGTCCATTAACACAAATTGCATCAGTATTTGTGGCTGCTTGG
AGAAATAGGCATATGGAATTGCCATCAGATTCATCACTACTTTATGAAATTGATGAC
AAATGTTTTGGTGAAGGACACAAAAAGAAGCCAAAATTGCCTCATAGCAAGGAATA
TCTATTCTTGGACAAGGCAGCCATTAAGGAAGATGGACTTGAAAGTAATGTTGTGAA
CAAGTGGAAAGTTTCAACATTAACAGATGTTGAAGAAGTAAAATTGTTATTCCGAAT
GTTACCAACTTGGGCCACAACCATTATGTTTTGGACTACCTATGCACAAATGACCAC
ATTTTCTGTTTCTCAAGCAACCACTATGGATCGTCACATCGGTAAATCCTTCGAAATC
CCGCCCGCCTCGCTCACCGCGTTCTTCGTTGGAGCCATCCTCTTGACCGTAATTGATC
GAGTCATCGTTCCGATTTGTCGACGTTTCGCTAACAAACGCCATGGACTCACCCCATT
GCAAAGAATTTTCATAGGTTTAATCCTTTCAATTATAGCCATGATTGCATCTGCCTTA
ACTGAGGTTAAAAGATTAAACACCGCCCACTTAAATGGGCTTACAAATGACCCAAAT
GCCACCATTCCATTAAGTGTATTTTGGCTAATTCCACAATTCTTGCTAATTGGGGCAG
GTGAAGCTTTTACATACATTGGCCAATTGGATTTTTTCTTAAGAGAATGTCCAAAAGG
TATGAAAACTATGAGTACTGGCCTATTTTTAAGTACCCTAAGATTAGGGTTTTTCTTT
AGTTCAATTTTGGTTACAATTGTACATATGGTTACTGGGACAAAACATCCATGGATA
GCTGATAATTTGAATCAAGGGAAGTTACATGATTTCTATTGGCTATTGGCAATATTGA
GTGTGTTGAACTTGATGGTTTTCTTGTATACGTCGAAAACGTACGTGTACAAGGAGA

AGAGGCTTGCAGAAATGGGGATTGAACTCGAGGACTCTGGACCAGTTTGTCACTAAG
ACGTACAGGATCACACTCTGTCACTACTAGAAAAATGTGAATTCAGTTTCACATTGG
AGAGTTATGTAATATAACAACTTTGTTTTATAATATGTACAAAAAGATGGAAAAGAG
CTTAGGTAATGAAAAATTATAGAGCTCTTTTCCAGTGTTTTTATTTTTTTTTCTTTACT
GTATATCATAATAATGTAATGCAATGCAATGCGATACATGAATTTCATGATTTGTGA
AAAAAAAAAAAAAAAAAAAAAA

**_NRT2.1:_** High affinity nitrate transporter protein - _Solanum lycopersicum_ (Tomato)
- 70% similar to Arabidopsis NRT2.4 (>AT5G60770.1 : ATNRT2.4 High Affinity Nitrate transporter).
- Response to nitrate. Lateral root development.
- Role in plant: nitrate transmembrane transporter activity
- Potentially coexpressed with: --
- Reference: Wang et al. (2001)
  - Primers Designed using Primer 3
  - F (5' -3'): TTCCTGTTACATTTTGTCATTTCC
  - R (5' – 3'): GGAACCAACACGCTTACCAC
  - Product length: 194 bp

DFCI – Tomato TC Report*: TC237840
GenBank accession number: AF092655

Nucleotide sequence:
TATTCTCAATACATTTCAAATCAATCATTTATAAAATTAACCAGTTATTTCCTCAATT
GAAGAAATGGCTGATGTAGAAGGATCACCGGGGAGTTCTATGCATGGAGTCACCGG
AAGAGAACCTGTTCTCGCTTTCTCCGTGGCTTCTCCAATGGTGCCTACGGATACCTCC
GCCAAATTTTCAGTACCGGTGGACACTGAACACAAGGCTAAACAATTTAAGTTTTAT
TCGTTTTCGAAGCCTCATGGACTTACGTTCCAGCTCTCCTGGATCTCCTTTTTCACTTG
TTTCGTTTCGACTTTTGCTGCTGCACCTTTAGTTCCTATTATTAGGGACAATCTTAATT
TGACAAAAATGGATGTTGGTAACGCTGGGGTTGCTTCCGTATCCGGAAGTATTTTAT
CTAGGCTTACGATGGGTGCGGTTTGTGATTTGTTGGGTCCAAGGTATGGGTGCGCTTT
TCTTATCATGTTGTCAGCGCCAACTGTTTTTTGTATGTCTTTTGTTTCATCCGCTGGTG
GCTACGTAGCTGTCCGGTTCATGATTGGGTTTTCGCTCGCAACGTTTGTGTCTTGTCA
ATATTGGATGAGTACTATGTTTAATAGTAAGATCATAGGGCTAGTGAACGGAACGGC
TGCTGGATGGGGTAATATGGGTGGAGGTGCAACTCAACTCATTATGCCACTTTTGTA
TGATATAATTCGAAGGGCGGGTGCAACTCCGTTCACTGCTTGGAGAATTGCATTTTTT
ATTCCTGGATGGCTTCATGTGGTGATGGGTATTTTAGTGTTGACTCTTGGCCAAGATT
TACCCGACGGAAATCGTGGCACTTTACAGAAGACGGGTACTGTTGCTAAAGATAAAT
TCGGTAACATATTGTGGTATGCTGCAACAAACTACAGGACATGGATCTTTGTTCTTCT
CTATGGATACTCTATGGGAGTTGAACTGTCAACAGACAACGTCATTGCTGAGTACTT
CTTCGACAGATTTGATCTAAAGCTTAGCACAGCGGGGATCATTGCTGCCACATTTGG
TATGGCTAACCTTTTGGCTCGACCATTTGGAGGATTTTCTTCTGATTACGCAGCAAAG
AAATTCGGTATGAGAGGGAGACTTTGGGTTTTGTGGATTTTACAAACACTTGGAGGA
GTATTTTGTGTTCTTTTGGGTCGTTCGAATTCTCTACCACTTGCGGTAACCTTTATGAT
CCTTTTCTCAATCGGAGCTCAAGCTGCTTGTGGTGCAACTTTTGGTATTATTCCATTC
ATTTCTCGACGATCGTTAGGAATTATAAGCGGAATGACAGGGGCAGGTGGAAATTTT
GGTTCTGGATTGACTCAATTGTTGTTTTTCACGAGCTCAAAGTACTCGACAGCGACAG
GGTTAACTTACATGGGATTCATGATCATAGGATGCACTCTTCCTGTTACATTTTGTCA
TTTCCCACAATGGGGAAGCATGTTTTTGCCACCAACAAAAGATCCAGTCAAGGGAAC
GGAAGAACATTATTATACTTCAGAGTACACAGAGGCCGAGAGGCAAAAAGGGATGC
ACCAAAACAGCTTGAAATTCGCTGAAAATTGCCGATCAGAGCGTGGTAAGCGTGTTG
GTTCCGCACCAACCCCACCAAATTTGACACCAAATCGTGTTTGATGATCTTTATGAGG
AATGGATAGTCTTGAATCTGTGATTTAAATTTAAGGTTCAATGTGCTGAGTCGTCTCA
ATAAGCAAAATCTATCTTGATTTTTCTTCTTTGTTTTTTTTTTATAATGATATTGCTTGT
TGATCTTTCCAGACAAATACCTTGAATCCACGAAGGTGTATGCTTTTTTTTTAATGAA
GTATATATAATATATTACTCATTGTGTATGTTTTCTATTGCTTTTTTTCAAAAGAATATT
CTATGGCCAATGGTGGTTGTGTTTTACTCTGTAGATTCAAAAGTGTATTATAATAAAA

CTCTTGACTTGTAAGAAGGGGACTGATCATTTATTCCAGTTGATTTATAGAAAGTTCG
TG

## Hormone Response Genes

**NOTE:** In addition to the genes listed below, the following genes, previously described above, also exhibit hormone responses: ***PR2, A-DOX1,CIPK24, MPK1, MPK3***, and ***PR1b***

<u>*ASA*</u>**:** Anthranilate synthase alpha subunit - *Solanum lycopersicum* (Tomato)
- 67% similar to Arabidopsis ASA1  (>AT5G05730:alpha subunit of anthranilate synthase.)
- Role in plant: catalytic activity; anthranilate synthase activity; anthranilate synthase complex.
- response to wounding, auxin biosynthetic process, response to ethylene stimulus, response to bacterium, aromatic amino acid family biosynthetic process
- Potentially coexpressed with: --
- Reference: Cartieaux et al. 2008

DFCI – Tomato TC Report*: TC234676
GenBank accession number:  BG791293

Nucleotide sequence:
TTTGCAAAATAGAACATGTTGACAATTATGGATCACCTTGAAGGAAGCAGAACAGA
GGAATTTGAGGAAGATCCAATAACCATTCCTAGTAGGATTATGGAAAAATGGAAAC
CTCAATGCATAAATGAGCTTCCTGAAGCATTTTGTGGAGGTTGGGTTGGTTTCTTCTC
ATATGATACTGTGCGTTACGTAGAGAAAAAGAAGCTACCTTTCTCAAATGCTCCAAT
GGATGATAGGAACCTTCCTGATCTTCATCTAGGACTTTATGATGATGTAATTGTGTTT
GATCATGTGGAAAAGAAAGCATTTGTCATACATTGGGTGCGGTTAGATCGCTTTGCT
TCAGTAGAGGAGGCCTACAATGATGGTACAACCAGATTAGAAGCTTTGTTGTCTAGA
GTACATGATATTGTACCTCCTACACTGGCTTCAGGGTCGATAAAACTTCATACTAGTC
TATTTGGTACTTCATTGAAAAATTCAACCATGACAAGCGAAGACTACCAGAAGGCTG
TTTTAAAGGCCAAGGAACATATCCTTGCTGGGGACATTTTCCAAATTGTTCTTAGTCA
ACGTTTTGAAAGACGAACCTTTGCAGATCCATTTGAAGTATACAGAGCACTAAGAAT
CGTAAATCCAAGTCCTTATATGACTTATCTACAGGCTCGGGGGGTGTATACTTGTTGCT
TCTAGTCCTGAAATTCTTACTCGAGTGAAGAAGAAACAGTTACCAATCGGCCCCTA
GCAGGGACTATTAGAAGAGGTAAGACACTTGAGGAAGATTATATGCTGGAAAATCA
ACTTTTGCACGACGAGAAACAGTGTGCAGAGCATATAATGCTGGTTGACTTGGGAAG
AAATGATGTTGGAAAGGTCTCTAAGCCTGGTTCGGTGAAAGTTGAGAAACTAACGAA
CATTGAACGGTATTCCCATGTCATGCACATCAGCTCTACGGTTACTGGAGAGCTACTT
GACCATTTGAGTAGCTGGGATGCTCTGCGTGCAGCCCTGCCTGTTGGAACCGTTAGT
GGAGCGCCTAAGGTAAAAGCCATGGAGCTAATTGATCAACTGGAAGTCACAAGGCG
TGGACCATACAGCGGTGGATTTGGAGGAATTTCCTTTACCGGAGAAATGGACATTGC
CTTAGCTTTGAGAACCATAGTATTTCCAACTGGAACGCGTTACGACACTATGTACTCG
TACAAGGATGTCGACAAGCGACGAGATTGGATTGCTTATCTCCAAGCTGGAGCAGGT
ATAGTGGCTGATAGTGACCCAGCTGATGAGCAAAATGAATGCGAAAATAAAGCTGC
AGCTCTTGTCCGTGCCATTGATCTTGCTGAGTCTTCATTTGTTGACAAATAATAGATG
CCGTCCTAGTGTCAGATTTTGTTTCGTCGATTATTTCGTTTCTTGTTGGATGTAGAAAG
TTCGTTTTCAATCTGAATAGTCCGTTATGTTGGGAGGTGACTGAAAATCCCCATCCAC
ACACACACTCACAATGTCATAGTCATTGCGTATGTTGGCTTCATCAATTTTAGCAGGG
TGTCACAAAGAGCCCAGAATTGGCATTGTGACTTATAGTTCTCTGGCTGTCAATTGAT
TTCAGTATTTGGCTTTTTTCGGGTTCTGTGGTTGTACCC

*IAA6*: Indole-3-Acetic Acid induced protein 6 - *Solanum lycopersicum* (Tomato)
- 73% similar and 60% similar to portions of Arabidopsis IAA6 (At1g52830: IAA induced protein 6).  Response to auxin stimulus.
- Role in plant: Senescence related, response to hormonal substances.
- Potentially coexpressed with: SAG12, IAA11
- Reference: http://atted.jp coexpression lists

DFCI – Tomato TC Report*: TC224851; TC199757
GenBank accession number: DB692221 (plus other ESTs)

Nucleotide sequence:
TTCTCTTGTAACTTTTTCTTTAAAAAAAGTTTTTTTCTTTTTCATCTCTTCCATAGTTTC
TTGAATTCTTGTAGAGAAATTCATCTTGTCTGTTTACTAATTTGTGCTCAACTTTCCAT
GTCTGTACCATTAGAACATGATTATATAGGTTTATCAGAACCTTCTTTAATGGAAAGA
AGTTCTGATAAGATTTCTTCTTCTTCTTCCTCTGTTCTAAACCTTAAGGAGACTGA
GCTGAGACTTGGGTTGCCTGGTTCTGAGTCTCATGGGGTTTCTCTTTTTGGCAAAGAT
TTGGACCCTTTAAGCAATTTTACATCAAGAACAAAAAGGGGTTTTTCTGATGCAATT
GATGCATCTGGAAAATCGGATTTGTCTATTAATTGCAGATCTGAAGCTGATAGGGAA
AACGGGAACTTGTTGTTTTCCCCAAAAAGAGGGAATGGAGGTTCAAACCCTGTTGAA
GAAAAAAAGCCTATCCCTCATACTTCAAAGGCACAAGTGGTAGGATGGCCACCAATT
AGATCATTCAGGAAAAATACACTGGCTACTAAGAAAAATGATGATGAAGGGAGAAC
AGGTTCAAGTTGCCTTTATGTTAAGGTTAGCATGGATGGTGCTCCATATCTGAGGAA
AGTTGATATCAAAACTTACAGTAACTATGCAGCGCTCTCATCAGCACTTGAAAAGAT
GTTCAGCTGCTTTAGTATTGGTCAGTGTGCCAGTGATAAGATTCCAGGGCAAGAGAA
GCTCAGTGAAAGTCACTTGATGGATCTTCTCAATGGTTCTGAAGTATGTGCTGACTTA
TGAGGACAAGGATGGTGATTGGATGCTAGTTGGCGATGTTCCTTGGGAGATGTTCAT
AGACTCATGCAAGAGATTGCGGATCATGAAGAGCTCACAGGCAATTGGGCTAGCTCC
AAGGGCCATAAATAAGTGCAAGAACCAAAATTAGTGACTGAAAGACTAACCGTCCA
AAGGGTTTCTACAACGTCAACCATCCTTTTTCTGCCCTGTTTGTATCTGGAATTAGAC
TAGATGTGTAGCATCCCCTGAAAGGGAGAGAGCTGGTTTAAGAAAATATAACCGGTC
AAAAATTGTACTGTGGCTAGTGTCTTTTGAGTGGCAATTTTTGCTTGCACATGCAACC
TGCAAGTTTATTTGCAAACATACATTAATTTTTAAGCATATAACCAACCAACTATTAT
TCTGATGAAACATAATAACTTCCAAGTTCTAACCTANAAAAAAAAAAAAAAAAAAAAA
AAAAANNACTTCGGGGGGGGGGCCCGGAACCCATTTCCCCCTAAAGGGAGTCGTATT
ACAATTCACGGGCCGTCTGTTTTAAAAANNGGGGGGGGGGGAAAGGCCCNGGGGTGG
GGTGGTTATATTAATTAAAAAAATCTGTGCGGTGACGACCACGTTGAGCGATTGAGG
GAAAAGGTGCGTGATGGGATTGTCGTGGCGAGAAGTTGGTTGTGGCCGTGGGGGGT
GATGTGTGAGTTAAGATGCATTTATATTTATTGAGTGGACGGTCACCGTGTACTTTGT
GTAATATTCATCTTTTTATTATACCGATGTGGGTGAGAAGGCGTGGAATTCCCGTCTA
TGATTGGTAAACCGCTGTGTCGTGTTTGTCGTCGAGTGAACTGTTTCTGGCTCTTTAA
TGGGAAGGCCGCAATTTTTAGGCTGATGGACTTCATGTAACGTATGTGATTCGAATG
AGAGGTTGCGAATCTGCGAGGGTGTGCTCGTGTTGAGCTTGCCTA

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*IAA11***:** Indole-3-Acetic Acid induced protein 11 - *Solanum lycopersicum* (Tomato)

- 62% similar to Arabidopsis IAA19 (At3g15540: IAA induced protein 19). Response to auxin stimulus.
- Role in plant: Senescence related**,** response to hormonal substances.
- Potentially coexpressed with: IAA6
- Reference: http://atted.jp coexpression lists

DFCI – Tomato TC Report*: TC244405;TC216372
GenBank accession number(s): AF022022, BP893356

Nucleotide sequence:
TGTGTGTGCTTACCGGAAAAAGAACAGTTTTAACGGACGTGAAGCTGAATCCAATAA
TAAAATGTACGTCAAAGTTAGCATGGATGGAGCACCGTTTTTGAGGAAAGTTGATTT
GAGTACTCATAAGGGTTATGATCAACTTGTTATGGCTCTTGAAAAACTCTTTGATTGC
TATGGAATTGGAGAAGCATTGGAGGATGCAGATAAGTCAGAGTTCGTTCCAATCTAT
GAAGACAAAGATGGAGATTGGATGCTTGTCGGCGATGTTCCATGGATAATGTTCAGT
GAATCATGCAAAAGGCTAAGGATCATGAAGAGATCAGAGGCAAAAGTGATAGGGCT
TGGAGCAAGAGACTTTCTCAAGGGAATGTCTCAAGAGAAATAGAGTGGATGATTCA
AGCTATATTTTTACATTAATATATAATTGTAATTGTAATCTTAGGTGTGATAAGACAC
CCTAGACTTAAAATCAATTTCTGATTTGATTTCCTCTTAATTAGCTTAATTATTGTAGT
AATCAGTACCTTATTTGCTCTTAAATGACAATTTTCGTATGTATAATAAGGCT

*SAG12*: KDEL-tailed cysteine endopeptidase (a.k.a. Senescence associated gene 12) - *Solanum lycopersicum* (Tomato)

- 63% similar to Arabidopsis SAG12 (At5g45890: Senescence-associated gene 12; encoding a cysteine protease influenced by cytokinin, auxin, and sugars). Cysteine-type peptidase activity, senescence associated vacuole, associated with leaf senescence, response to ethylene stimulus, associated with aging.
- Role in plant: Senescence related, response to hormonal substances.
- Potentially coexpressed with: IAA6
- Reference: Kumar et al. (2004)

DFCI – Tomato TC Report*: TC217581
GenBank accession number: DB717566 (plus other ESTs)
*NOTE:* Different from GenBank EST used in Kumar et al. 2004: AI776170.  Using this EST in BLAST [at http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/Blast/index.cgi] produces TC Report# **TC195364** which codes for a Phytothora resistant protease)

Nucleotide sequence:
ACCCATTGCTTCATAACATCATAATTTTATTTCAATATTTTCACCACTTCTTTTATTAT
TGTTGAGTGTTATTTCTTCCATAAGCAATCAATCACTTTGTTTCATTTATCTATAAAAG
GCCCTCTTCATAGTCATCTAAAATAATCACATCTTGAGTCTCTCTATCTTTCAAAATG
AAGAAGTTGTTTCTAGTTCTTTTCACCTTAGCTTTGGTACTTAGGCTCGGGGAGAGTT
TCGATTTCCACGAGAAAGAATTAGAGACTGAGGAAAAATTCTGGGAGTTGTATGAG
AGATGGAGAAGCCATCACACTGTATCGAGGAGCCTTGACGAGAAACACAAGAGGTT
TAATGTGTTTAAGGCTAATGTTCATTATGTTCACAACTTCAACAAGAAGGATAAGCC
TTATAAGTTGAAACTGAATAAGTTTGCAGACATGACTAACCATGAATTCAGACAGCA
TTATGCTGGTTCTAAGATTAAGCATCATCGTACTTTGCTTGGAGCTTCACGAGCAAAT
GGAACTTTCATGTACGCCAACGAGGATAATGTCCCTCCTTCTATTGACTGGAGGAAG
AAAGGTGCTGTCACTCCTGTCAAAGATCAAGGACAGTGTGGAAGTTGCTGGGCATTT
TCAACTGTGGTCGCGGTAGAGGGGATAAACCAAATCAAAACAAAGAAATTAGTATC
TTTGTCGGAGCAAGAACTTGTTGACTGTGACACTACAGAAAACCAAGGATGCAATGG
AGGATTGATGGACCCGGCATTTGACTTCATCAAGAAGAGGGGCGGCATCACAACAG
AGGAGAGGTATCCTTATAAGGCTGAAGATGACAAGTGTGACATTCAAAAGAGGAAT
ACTCCGGTGGTTTCAATTGACGGACACGAGGATGTTCCTCCTAATGATGAGGATGCA
CTGCTTAAAGCAGTAGCCAACCAGCCTATTTCTGTAGCTATAGACGCTTCAGGTTCTC
AGTTCCAGTTCTACTCTGAGGGCGTATTCACCCGGAAAAGTGGTACTGAATTGGAAC
ATGGGGTGGCTATTTGGGGGGATGGGCACACCGTCGATGGAACCCAATAATGGGATT
GTGAGAAATCCTGGGGGAACTGATTGGGGAAAAAAGGATACCTTAAAATGCCCCC
CAGGTTGACCCCTAAAAGAGGGTTGTGGGTATAGAAATGAAACCTCTCTCCCCTTAA
AAATTCAGAAAACCTTAGGGGCCTCCTGCGCCCCACTAAGGATAAATTTAATT

# APPENDIX B

# SUPPLEMENTAL CORRELATION TABLES FOR CHAPTER 3

**Table B1.** Pearson's correlations showing relationships between $\log_2$ transformed relative expression ratios of selected tomato genes, as well as plant health characteristics (height, dry biomass, and chlorophyll content) and Mehlich III extracted nutrients and chemical parameters. Only genes that showed significant differences in gene expression due to soil management are presented. Asterisks (*, **, ***) indicate significant trealtionships at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

| Soil Parameter | ——Differentially expressed genes—— | | | | | ——Plant health characteristics—— | | |
|---|---|---|---|---|---|---|---|---|
| | GS1 | ChiB | GluA | Osm | PR1b | Height | Biomass | Chlorophyll |
| P | 0.36 | -0.50* | -0.63** | -0.48* | -0.34 | -0.08 | 0.01 | -0.42 |
| K | 0.33 | -0.14 | -0.08 | -0.11 | 0.42 | 0.49† | 0.89† | 0.59† |
| Ca | 0.32 | -0.36 | -0.57* | -0.49* | -0.36 | -0.22 | -0.19 | -0.56* |
| Mg | 0.46 | -0.50* | -0.47* | -0.38 | -0.28 | 0.04 | 0.16 | -0.31 |
| Zn | 0.37 | -0.39 | -0.29 | -0.18 | -0.16 | 0.13 | 0.20 | -0.14 |
| Cu | 0.13 | 0.20 | 0.41 | 0.39 | 0.33 | 0.16 | 0.03 | 0.09 |
| Mn | -0.56* | 0.14 | -0.26 | -0.14 | -0.29 | -0.40 | -0.41 | -0.22 |
| Na | 0.22 | -0.27 | -0.07 | -0.01 | 0.01 | 0.22 | 0.21 | -0.01 |
| Fe | -0.04 | -0.23 | -0.57† | -0.43 | -0.29 | -0.27 | -0.34 | -0.53 |
| Al | -0.34 | 0.29 | -0.05 | 0.01 | -0.43 | -0.63** | -0.69** | -0.39 |
| Ni | 0.19 | -0.39 | 0.07 | -0.24 | 0.22 | 0.53* | 0.36 | 0.08 |
| Total N | 0.08 | -0.08 | 0.12 | 0.07 | 0.65** | 0.64** | 0.82*** | 0.68** |
| NH₄-N | 0.15 | -0.19 | -0.02 | 0.04 | 0.18 | 0.41 | 0.59† | 0.40 |
| NO₃-N | -0.19 | 0.16 | 0.22 | 0.12 | 0.46 | 0.44 | 0.62† | 0.63† |
| Total C | 0.55† | -0.36 | -0.17 | -0.15 | 0.25 | 0.48* | 0.73** | 0.29 |
| POXC | 0.52† | -0.55† | -0.25 | -0.37 | 0.19 | 0.52* | 0.68** | 0.21 |
| pH | 0.30 | -0.53† | -0.61† | -0.50† | -0.51† | -0.18 | -0.18 | -0.56† |
| CEC | 0.44 | -0.25 | 0.07 | -0.07 | 0.46 | 0.42 | 0.45 | 0.22 |
| Base Sat. | 0.46 | -0.43 | -0.59** | -0.51* | -0.32 | 0.03 | 0.24 | -0.31 |

† Relationships not interpretable due to clustering of data or cases with high leverage

**Table B2**. Pearson's correlations showing relationships between $\log_2$ transformed relative expression ratios of selected tomato genes and plant nutrient contents and health parameters. Only genes that showed significant differences in gene expression due to soil management are presented. Asterisks (*, **, ***) indicate significant trealtionships at $p \le 0.05$, $p \le 0.01$ and $p \le 0.001$, respectively.

| Nutrient/Health Parameter | GS1 | ChiB | GluA | Osm | PR1b |
|---|---|---|---|---|---|
| Ca | -0.33 | 0.11 | -0.38 | -0.15 | -0.37 |
| Cd | -0.42 | 0.26 | 0.09 | -0.02 | 0.01 |
| Co | 0.06 | 0.23 | -0.14 | 0.17 | 0.22 |
| Cr | -0.51† | -0.08 | 0.01 | -0.26 | -0.06 |
| Cu | 0.14 | 0.09 | 0.07 | 0.05 | -0.40 |
| Fe | -0.46 | 0.16 | 0.23 | 0.09 | -0.11 |
| K | -0.24 | -0.19 | 0.04 | -0.27 | 0.06 |
| Mg | -0.42 | -0.05 | -0.25 | -0.18 | -0.57* |
| Mn | -0.43 | 0.30 | 0.29 | 0.21 | 0.46 |
| Mo | -0.02 | -0.46 | -0.73† | -0.60† | -0.69† |
| Ni | -0.69** | 0.19 | 0.06 | 0.06 | -0.10 |
| P | 0.10 | -0.62** | -0.50* | -0.72*** | -0.76*** |
| Zn | -0.04 | 0.16 | 0.13 | 0.07 | -0.29 |
| Kjeldahl N | -0.67† | 0.30 | 0.24 | 0.26 | 0.22 |
| Plant Height (cm) | 0.31 | -0.36 | 0.46 | -0.19 | 0.67** |
| Chlorophyll (mg $g^{-1}$) | 0.03 | 0.39 | 0.32 | 0.51* | 0.61** |
| Dry Biomass (g) | 0.35 | -0.01 | 0.07 | 0.08 | 0.61** |

† Relationships not interpretable due to clustering of data or cases with high leverage

**Table B3.** Pearson's correlations showing relationships between $\log_2$ transformed relative expression ratios of selected tomato genes and concentrations or relative abundances of soil microbial biomarkers. Only genes that showed significant differences in gene expression due to soil management are presented. Asterisks (*, **, ***) indicate significant realtionships at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

| Biomarker or Group | GS1 | ChiB | GluA | Osm | PR1b | GS1 | ChiB | GluA | Osm | PR1b |
|---|---|---|---|---|---|---|---|---|---|---|
| | —— vs. Biomarker Concentrations—— | | | | | —— vs. Biomarker Relative Abundance—— | | | | |
| Ergosterol | 0.44 | -0.40 | -0.19 | -0.25 | 0.12 | -- | -- | -- | -- | -- |
| 14:0 | 0.61† | -0.44 | -0.19 | -0.28 | 0.17 | -0.18 | 0.22 | 0.10 | 0.12 | -0.01 |
| i15:0 | 0.49† | -0.44 | -0.32 | -0.28 | 0.03 | -0.56* | 0.34 | -0.17 | 0.19 | -0.40 |
| a15:0 | 0.49† | -0.41 | -0.29 | -0.26 | 0.06 | -0.41 | 0.29 | -0.29 | 0.15 | -0.44 |
| 15:0 | 0.52† | -0.39 | -0.22 | -0.22 | 0.13 | -0.41 | 0.52† | -0.01 | 0.46 | -0.16 |
| i16:0 | 0.62† | -0.28 | -0.10 | -0.12 | 0.36 | 0.048 | 0.41 | 0.28 | 0.38 | 0.34 |
| 16:1ω7c | 0.51† | -0.51† | -0.26 | -0.37 | -0.13 | -0.30 | 0.03 | 0.01 | -0.10 | -0.60† |
| 16:1ω5c‡ | 0.52† | -0.37 | 0.07 | -0.19 | 0.42 | -0.10 | 0.17 | 0.34 | 0.19 | 0.25 |
| i17:1ω7c | 0.53† | -0.46 | -0.32 | -0.32 | -0.01 | -0.19 | 0.07 | -0.38 | -0.09 | -0.55* |
| 10me16:0 | 0.51† | -0.38 | -0.22 | -0.20 | 0.06 | -0.52* | 0.51* | 0.19 | 0.44 | -0.16 |
| i17:0 | 0.62† | -0.35 | -0.17 | -0.18 | 0.23 | -0.04 | 0.35 | 0.11 | 0.28 | 0.07 |
| a17:0 | 0.65† | -0.32 | -0.14 | -0.18 | 0.33 | 0.06 | 0.35 | 0.15 | 0.26 | 0.21 |
| cy17:0 | 0.35 | -0.41 | -0.24 | -0.33 | -0.05 | -0.42 | 0.16 | 0.02 | 0.02 | -0.33 |
| 10me17:0 | 0.48† | -0.54† | -0.03 | -0.53† | -0.07 | 0.20 | -0.39 | 0.16 | -0.51† | -0.27 |
| 18:2ω6c‡ | 0.32 | -0.35 | -0.19 | -0.19 | -0.02 | 0.15 | -0.27 | -0.19 | -0.11 | -0.06 |
| 18:3ω3 | 0.38 | -0.40 | -0.03 | -0.49† | 0.28 | 0.12 | -0.27 | 0.08 | -0.47* | 0.16 |
| 18:1ω7c | 0.58† | -0.54† | -0.22 | -0.40 | 0.03 | 0.51* | -0.69** | -0.49* | -0.76*** | -0.46 |
| 18:1ω5c | 0.37 | -0.23 | -0.06 | -0.14 | 0.29 | 0.31 | -0.17 | -0.04 | -0.13 | 0.32 |
| 10me18:0 | 0.53† | -0.27 | -0.17 | -0.14 | 0.28 | -0.43 | 0.67** | 0.21 | 0.52 | 0.24 |
| cy19:0 | 0.61† | -0.24 | -0.01 | -0.07 | 0.45 | 0.02 | 0.35 | 0.32 | 0.34 | 0.42 |
| Actinomycete | 0.56† | -0.41 | -0.19 | -0.26 | 0.10 | -0.48* | 0.49* | 0.24 | 0.38 | -0.13 |
| Bacteria | 0.59† | -0.45 | -0.23 | -0.30 | 0.09 | -0.23 | 0.27 | 0.04 | 0.13 | -0.37 |
| Gram+ Bacteria | 0.55† | -0.40 | -0.26 | -0.24 | 0.15 | -0.40 | 0.45 | 0.00 | 0.33 | -0.15 |
| Gram- Bacteria | 0.56† | -0.53* | -0.23 | -0.40 | 0.01 | 0.20 | -0.44 | -0.33 | -0.58* | -0.63** |
| Total FAMEs | 0.53† | -0.43 | -0.18 | -0.27 | 0.13 | -- | -- | -- | -- | -- |

† Relationships not interpretable due to clustering of data or cases with high leverage.

‡ The FAMES 16:1ω5c and 18:2ω6c are signature FAMES for arbuscular mycorrhiza and fungi, respectively.

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**Shawn T. H. Lucas**

## EDUCATION

*Ph.D., Soil Science. August 2013,*  University of Kentucky, Lexington, KY
*Ph.D. Soil Science. (Degree not completed, Transferred to UK)*, The Ohio State University, Columbus, OH
*M.S., Soil Science.  Dec. 2004,*  University of Maryland, College Park, MD
*B.S., Microbiology, 1994; B.A. Psychology, 1994,*  University of Rochester    Rochester, NY

## TEACHING AND PROFESSIONAL EXPERIENCE

| | |
|---|---|
| Aug. 2009 – Dec. 2010 | *Teaching Assistant.* University of Kentucky, Lexington, KY. |
| Aug. 2004 – Aug. 2006 | *Teaching Assistant.* Ohio State University, Columbus, OH. |
| Aug. 1999 – May 2004 | *Teaching Assistant.* University of Maryland, College Park, MD. |
| June 1996 – Aug. 1999 | *Microbiology Editor.*  Cambridge Scientific Abstracts, Bethesda, MD. |
| Sept. 1994 – June 1996 | *Laboratory Technician IV*, University of Rochester Medical Center, Dept. of Biophysics, Rochester, NY |
| June 1992 – Aug.1994 | *Laboratory Technician II*, University of Rochester Medical Center, Dept. of Dental Research, Rochester, NY |

## PROFESSIONAL SOCIETY AFFILIATIONS

- American Society of Agronomy (member since 2012)
- Soil Science Society of America (member since 2003)

## AWARDS

- 2006, The Ohio State University: Gamma Sigma Delta Agricultural Honor Society.
- 2005, The Ohio State University: Frank L. Himes Honorarium for outstanding teaching.
- 2004, Univ. of Maryland: Dept. of Natural Resource Sciences, Outstanding Graduate Student Award.
- 2003, Univ. of Maryland: Distinguished Teaching Assistant Award.

## PEER REVIEWED PUBLICATIONS

**Lucas, S.T.**, and R.R. Weil. 2012. Can a Labile Carbon Test be Used to Predict Crop Responses to Improved Soil Organic Matter Management?  Agronomy Journal 104:1160–1170.